THE
AMERICAN NATURALIST

VOL. XLIX *February, 1915* No. 578

CYCLES AND RHYTHMS AND THE PROBLEM OF "IMMORTALITY" IN PARAMECIUM

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THE recent brilliant work of Woodruff and Erdmann has thrown a flash of light upon the old question of age and death in protozoa and upon the problem of the significance of conjugation. The long, successful cultivation of *Paramecium aurelia* by Woodruff led Jennings to say:

> The work of Woodruff demonstrates that the very limited periods within which Maupas and Calkins observed degeneration has no significance for the question as to whether degeneration is an inevitable result of continued reproduction without conjugation. In other words, it annihilates all the positive evidence for such degeneration, drawn from work on the infusoria. It justifies the statement that the evidence is in favor of the power of these organisms to live indefinitely, if they are kept under healthful conditions. It shows that Weismann was correct in what he meant by speaking of the potential immortality of these organisms.[1]

The same work of Woodruff led Minot to say in the course of his German lectures:

> Quite conclusive as to the absence of senescence are the experiments of L. L. Woodruff, who has maintained a pedigreed race of *Paramecium* for five years without conjugation.[2]

Woodruff also makes the statement in different publications that the cells of his pedigreed race of *Paramecium*

[1] "Age, Death and Conjugation in the Light of Work on Lower Organisms," *Pop. Sci. Mo.*, June, 1912, p. 568.

[2] "Modern Problems of Biology," 1913, p. 62.

aurelia possess the potentiality to perpetuate themselves indefinitely by division under proper environmental conditions. In short, his results have given almost the only experimental evidence in support of the view, advocated by Weismann, that protozoa are potentially immortal.

The importance of this generalization and the deductions from it are self evident, and it is unfortunate that so many should have advanced it before the life history of *Paramecium aurelia* was fully known. Woodruff is to be congratulated, however, in that he, with Miss Erdmann, has now worked out stages in the life history of this organism which go far in clearing up the discrepancy between his results and those obtained by Maupas and his followers.

Woodruff has carried on his pedigreed race of *Paramecium aurelia* for more than seven years with a fairly uniform division rate, subject, however, to occasional and periodic fluctuations which he calls rhythms. These correspond roughly to what I have termed cycles which end in depression periods and, unless stimulated, by death; in rhythms, however, Woodruff maintains, there is no evidence of depression.[3] Recently Woodruff finds from a careful study of material fixed during the low periods of his division rate rhythms that there takes place a complete nuclear reorganization, after which the organisms continue to live with renewed vitality as shown by the ascending division rate. This process consists in the disintegration and probable absorption in the cytoplasm of the old macronucleus, one or more divisions each of the old micronuclei, degeneration of some of the products of these divisions, and the ultimate reformation of functional macronuclei and micronuclei from others. These are, in essence, the important new facts which cytological study has revealed in the life history of *Paramecium aurelia,* and some evi-

[3] I would like to suggest to Professor Woodruff that he work out the death rate, the data for which he can undoubtedly obtain from his records. I venture to predict he will find that the death rate rises with the decline of the division rate and during the low sweep of the rhythms.

dence is further adduced to show that similar processes occur in *Paramecium caudatum*.

While there is little to criticize in regard to the facts as described for this remarkable process, there is room for difference of opinion in regard to the conclusions which Woodruff and Erdmann draw from them. In addition to what Hertwig has already written about this work, I would call attention particularly to their conclusions concerning endomixis, parthenogenesis, conjugating and non-conjugating lines, the life cycle, and the potential immortality of *Paramecium*.

In regard to endomixis the authors state:

> Since the process results in the dissemination of the material from the old macronucleus and the so-called reduction micronuclei in the cell, it gives the opportunity for a rearrangement of the molecular constitution of the cell. This involves a more profound intermingling of nuclear and cytoplasmic substances than is possible during the typical vegetative life of the cell. *Since this intermingling occurs within a cell we term this reorganization process endomixis.* Endomixis is followed by a slight acceleration of cell phenomena and a new rhythm is initiated.[4]

Further on they add:

> We would therefore put emphasis on molecular rearrangement as the result common both to endomixis and to conjugation.[5]

Nearly forty years ago Engelmann interpreted conjugation in much the same way as a process of reorganization of the cell:

> . . . die Conjugation der Infusorien leitet nicht zu einer Fortpflanzung durch 'Eier,' 'Embryonalkugeln' oder irgend welche andere Keime, sondern zu einem eigenthümlichen Entwickelungsprocess der conjugirten Individuen, den man als Reorganization bezeichnen kann.[6]

In several places in the same publication Engelmann speaks of physical and chemical changes as accompanying

[4] Woodruff and Erdmann, "A Normal Periodic Reorganization Process without Cell Fusion in Paramecium," *Jour. Exp. Zool.*, Vol. 17, No. 4, 1914, p. 491. The italics are in the original.

[5] *Ibid*, p. 491.

[6] "Ueber Entwickelung und Fortflanzung von Infusorien," *Morph. Jahrb.*, 1, 1876, p. 628.

conjugation. A similar interpretation was given by Calkins:

> . . . it is now a well-known fact that in this process of reorganization the old macronucleus fragments and ultimately disappears in the cytoplasm. This disappearance must give rise to a great increase in the nucleo-protein content of the cell, therefore to a new chemical composition of the cell as a whole. We have recently shown that, under certain conditions, nucleo-proteins (especially the purines) have a markedly stimulating effect on the rate of cell division.[7]

Now such intermingling is no more characteristic of this process of asexual reorganization than it is of the reorganization following conjugation. In both cases, as Woodruff and Erdmann show, reorganization is effected by the physical and chemical change of the old macronucleus and portions of the old micronucleus or micronuclei. The sole difference in these processes of reorganization is not to be found in the molecular rearrangement of the cell, but, as Woodruff and Erdmann state, in the presence after conjugation of a syncaryon and the nuclei derived from it. This difference, however, does not amount to much in closely related pairs in conjugation. Several observers have shown that closely related individuals, even sister cells, of *Paramecium* may conjugate, and I have followed out through 360 generations the history of such an endogamous exconjugant from a pair which came from the same ancestral cell not more than ten days prior to conjugation. There can not be a great difference in the syncaryon resulting from such a union, over the functional micronucleus had it undergone asexual endomixis. In other words, the excellent term endomixis does not indicate phenomena peculiar to asexual reorganization in *Paramecium*, but applies equally well to the process of reorganization following conjugation. The terms asexual endomixis and sexual endomixis may serve to distinguish the process of intermingling during parthenogenesis and after conjugation, respectively.

[7] "The Paedogamous Conjugation of *Blepharisma undulans*," *Jour. Morph.* Vol. 23, 1912, p. 685.

Woodruff and Erdmann limit the application of the term endomixis to the process of reorganization without conjugation:

> We therefore have employed a new term "endomixis" for the reorganization process in Paramecium, in preference to parthenogenesis which Hertwig applied when he incidentally noted some isolated stages of the nuclear phenomena which we have elucidated.[8]

A new name can not alter the significance of a process or phenomenon. Parthenogenesis, in its broad sense, is the development of an individual from an egg without fertilization. In the same sense that a *Paramecium* ex-conjugant develops into a new individual, so does a *Paramecium* after this process termed endomixis. Woodruff and Erdmann say:

> In parthenogenesis there is a chromatin reduction which occurs and is compensated for either in the egg itself or in some later period of the life cycle of the race.[9]

The authors are not very happy in selecting this feature as distinguishing parthenogenesis from asexual endomixis, for in most cases of recognized parthenogenesis in metazoa chromatin reduction plays no part; for example, the majority of parthenogenetic eggs give off only one polar body, thus retaining in the egg the diploid number of chromosomes; others, notably the aphids and phylloxerans, do not undergo synapsis or chromatin reduction; some others it is true, give off both polar bodies and develop with the haploid number of chromosomes as is the case in bees (males), and in artificial parthenogenesis. As to the significance of parthenogenesis neither polar body formation nor chromosome reduction furnishes the key, for in many cases the eggs are predestined to parthenogenetic development long before the polar body nuclei are formed.

In regard to the reducing divisions of the chromosomes in *Paramecium* we know very little. Evidence has been adduced to indicate that the chromosomes are divided

[8] *Ibid.*, p. 493.
[9] *Ibid.*, p. 492.

longitudinally in both the first and the second divisions of the maturation process. The significance of the third division is as obscure in *Paramecium* as maturation is in some metazoan hermaphrodites.

In parthenogenesis, finally, we are dealing with a biological phenomenon, not with an interpretation of parthenogenesis by Winkler or Strasburger or any other individual, and to interpret this highly significant phenomenon in *Paramecium* solely in the light of such definitions, as Woodruff and Erdmann do (p. 493), does not carry conviction, nor does it conceal the real significance of the phenomenon. Asexual endomixis in *Paramecium* is parthenogenesis and nothing else, as Hertwig originally maintained in connection with these same phenomena. Nor, except for the protozoa, is it a "new type of parthenogenesis" for, if we accept conjugation as equivalent to fertilization, its analogue is shown by the majority of parthenogenetic eggs.

In regard to conjugating and non-conjugating races of *Paramecium*, Woodruff and Erdmann state:

> *Thus it is proved that both the reorganization process and conjugation are potentialities of the same race*—and therefore there is no evidence for the view of Calkins ('13) that conjugating and non-conjugating races of Paramecium exist, or that "apparently some paramecia are potential germ cells, others are not."[10]

This is rather a sweeping generalization to draw from one pedigreed line in which conjugating animals appeared only after six years in culture. If every *Paramecium* is a potential germ cell, why was it that no pairs of conjugating *aurelia* were found during these six years? Or, in Calkins and Gregory's observations on the first 32 cells and the pure lines arising from them, all from a single ex-conjugant, why was it that all lines from one quadrant gave epidemics of conjugation whenever the test was made during a period of six months, while all other lines from the remaining three quadrants failed to give a single pair when tested under identical conditions?

[10] *Ibid.*, p. 490. Italics in the original.

Woodruff and Erdmann maintain that "under just the proper conditions" conjugation will occur; this, of course, can not be denied, but the fact that under the same conditions some lines will conjugate while others will not shows a physiological difference between them which can not be gainsaid. I have no paternal jealousy whatsoever in regard to the terms "conjugating lines" and "non-conjugating lines," and am entirely willing to accept in their place any terms which indicate the physiological difference that I wished to express. I know of no terms that express the conditions adequately. Substitute for them, if more suitable, such expressions as "always ready to conjugate" and "rarely ready to conjugate." Our observations on the 32 lines certainly justify the statement that some lines in regard to conjugation, were always ready, while others were rarely ready. Woodruff and Erdmann have paid no attention to the physiological conditions which the (perhaps unfortunate) expressions "conjugating lines" and "non-conjugating lines" were meant to express. It is true that after ten months all but four of the so-called non-conjugating lines each furnished a few pairs of conjugating individuals, just as Woodruff's line did after six years, facts which show that the terms "conjugating lines" and "non-conjugating lines" as applied to races of *Paramecium,* if used at all, should be used only in respect to relative intensity of conjugating power. In this sense Woodruff's race is a non-conjugating race. We have found, furthermore, that conjugating lines have a lower vitality as measured by the division rate, and a much higher death rate, than do non-conjugating lines, all but four of the eight lines from the conjugating quadrant dying out within three months as against four of the twenty-four lines of the non-conjugating quadrants, while at the end of twenty months only one conjugating line was alive and sixteen non-conjugating lines, a mortality of 87.5 per cent. for the former and 33.3 per cent. for the latter. In *Paramecium* it is conceivable that lines with a high conjugating

power have a less well developed power of asexual endomixis than do lines that are relatively sterile, and this, correlated with their reduced vitality, if conjugation were prevented, would account for the death of all pedigreed races prior to Woodruff's, which, as Woodruff and Erdmann now show, has a high power of asexual endomixis. We are still justified, I believe, in maintaining the statement—modified now by their description of asexual reorganization—as quoted by Woodruff and Erdmann:

> Woodruff's *Paramecium aurelia* is evidently a *Paramecium Methuselah* belonging to a non-conjugating line the life history of which is not known in any case.[11]
>
> It is clear that the cycle emphasized by Maupas, Calkins and others is merely a phantom which has continually receded as each successive investigator has approached the problem with improved culture methods until it has vanished with Woodruff's race of (so far) 4,500 generations. What remains then is the rhythm and in the light of the present study, which demonstrates the underlying cytological phenomena of which it is an outward physiological expression, the whole problem takes on a new aspect. The cell automatically reorganizes itself periodically by a process which, in its main features, simulates conjugation—but without a contribution of nuclear material from another cell. Therefore it is evident (as has been shown by this culture) that the formation of a syncaryon, whose components are derived from two cells, is not necessary for the continued life of the cell—it has an internal regulating phenomenon which is entirely adequate to keep it indefinitely in a perfectly normal condition.[12]

Here we are brought up sharply to face the question which every student of pedigreed infusoria since Maupas has tried to solve. Woodruff and Erdmann conclude from their observations that old age and natural death do not occur in *Paramecium* and that the so-called "cycle" is non-existent. I would draw from their observations exactly the opposite conclusions, viz., that the one apparent exception among pedigreed races, to the rule of depression and natural death in the absence of conjugation or its equivalent, is now removed, and that Woodruff's culture is no more than a long series of cycles.

[11] *Ibid.*, p. 429.

[12] Woodruff and Erdmann, p. 489.

We understand by a "cycle," in the sense with which the term was first employed by Calkins, a more or less periodic alternation of high and low vitality as measured by the division rate. The lowering division rate indicates the approach of a period of depression which was interpreted as the equivalent of old age in metazoa, since it indicates a weakening in the chain of vital activities and ends in death unless conjugation or its equivalent is permitted. No one since Maupas, so far as I am aware, has attempted to limit a cycle in terms of definite numbers of generations or definite lengths of time. In 1904 I stated:

> The well-marked cycles, therefore, with periods of depression which demanded stimulation of a decided character, were apparently of *six months* duration, while intermediate cycles of less importance were about three months long. . . . During the first three cycles the number of generations was nearly the same (200, 198, and 193, respectively), the last, on the other hand, was much less, the individuals dividing only 126 times.[13]

The period of six months, more or less, or $200 \pm$ generations were not regarded as measures of the cycle, and it was understood at that time that conjugation or its equivalent always inaugurates a new cycle. Woodruff in 1905 introduced the term "rhythm" to designate the lesser periodic fluctuations which I had called "intermediate cycles." Since the entire substance of the much-discussed problem of immortality in infusoria is bound up with this question of the cycle, it is necessary to analyze the so-called rhythms of Woodruff to see how they agree with or differ from the so-called cycles. In *Paramecium* the cycle consists of the history of a bit of protoplasm in an ex-conjugant and its progeny from which conjugation or its equivalent is excluded, until natural death of the entire race ensues. If conjugation or its equivalent occurs the old cycle is abandoned and a new one is started, and there must be as many new cycles as there are times when conjugation or its equivalent takes place. It is imma-

[13] "Studies on the Life History of the Protozoa," IV. *Jour. Exp. Zool.*, Vol. I, 1904, p. 424.

terial, furthermore, whether such conjugation occurs between individuals of the same race, or between individuals of diverse ancestry, the effect is the same in putting off ultimate weakness and death. With repeated conjugations in such a race the ultimate death may be postponed indefinitely, and this was the argument on which Weismann's revised theory of potential immortality was based.

Now it is exactly the same with Woodruff's rhythms. He finds in his long culture repeated instances of ascending and descending division rates in fairly regular alternate succession. The descending division rate is stopped by an "internal regulatory phenomenon, endomixis."[14] Woodruff and Erdmann, while showing that endomixis is different from conjugation in the absence of a syncaryon, apparently accept it as equivalent to conjugation in connection with vitality of the protoplasm:

> Endomixis and conjugation may occur simultaneously in different animals of the same culture, thus strongly suggesting that the same *general* conditions lead to both phenomena—one animal meeting the conditions one way and another by the other, and that *both phenomena fill essentially the same place in the economy of life of Paramecium aurelia.*[15]

Again they say:

> Endomixis does initiate a new rhythm in the life history of Paramecium, *i. e.*, a period of increased metabolic activity and therefore of reproductive activity, and since its fundamental morphological features are almost identical with those preliminary to the formation of the stationary and migratory micronuclei in conjugation, it lends strong support to the view that the dynamic aspect of conjugation is not absent.[16]

Throughout the long period of seven years the *Paramecium aurelia* protoplasm without conjugation: "has undergone endomixis frequently, undoubtedly on the average once each month" (*ibid.*, p. 495). Hertwig has already shown, as I do above, that asexual endomixis is parthenogenesis, and if, in connection with the problem of vitality, this is equivalent to conjugation, then we are

[14] *Ibid.*, p. 497.

[15] *Ibid.*, p. 492; the italics at the end are mine.

[16] *Ibid.*, p. 496.

justified in saying that throughout the seven years Woodruff's *Paramecium* has undergone the equivalent of conjugation on the average once each month, and if it is equivalent to conjugation, then his long culture of more than 4500 generations has no bearing on the question of old age and natural death in *Paramecium*.

Nothing in this work of Woodruff and Erdmann seems more clearly and forcibly demonstrated than that the cycle, this "phantom" of many investigators, resolves itself into a demonstrated fact, and that Woodruff's "rhythm" and Calkins's "cycle" are but different names for the same phenomenon. If natural death is a necessary end to justify our use of the term "cycle," we may ask the pertinent question: What happened to those individuals which did not undergo asexual endomixis in Woodruff's long culture? If they died, does not this fact indicate the end of a cycle? If they underwent parthenogenesis, the equivalent of conjugation, does not this fact indicate the beginnings of new cycles? If they continued to live without reorganization, evidence for which has never been given by Woodruff, then there would be some justification for our authors' conclusion. To argue that it is the same race which continues after asexual endomixis is to use the same argument that Weismann used unsuccessfully, viz., that an ex-conjugant is the same old individual since no corpse has been formed and therefore the infusoria are immortal.

The frequent statement made by Woodruff that his long culture sustains the view that old age and need of conjugation are not necessary attributes of living matter are contradicted by these later results. For example, he states in 1913:

> Diese Untersuchung hat uns gezeigt, dass, unter günstigen äusseren Umständen, das Protoplasma der zuerst isolierten Zelle mindestens die Potenz hatte, ähnliche Zellen bis zu einer Zahl von 2^{3340} und eine Masse Protoplasma von mehr als 10^{1000} mal der Masse des Erdballes zu erzeugen. Dieses Resultat, glaube ich, bestätigt unzweifelhaft die Annahme, dass das Protoplasma einer einzigen Zelle unter günstigen

äusseren Umständen ohne Hilfe von Konjugation oder einer künstlichen Reizung imstande ist, sich unbegrenzt fortzupflanzen und zeigt ferner in klarer Weise, dass das Altern und das Befruchtungsbedürfnis nicht Grundeigenschaften der lebendigen Substanz sind.[17]

I am entirely in sympathy with Hertwig when he says, in connection with this citation:

Nach meiner Ansicht sind die Resultate, zu denen in den unseren Auseinandersetzungen zum Ausgangspunkt dienenden Artikel Woodruff gemeinsam mit Rhoda Erdmann gelangt ist, mit den hier zitierten Sätzen unvereinbar.[18]

The discovery of parthenogenesis in the life cycle of *Paramecium aurelia* by Woodruff and Erdmann clears up the obscurity which has involved all theoretical discussions following pedigreed culture work with infusoria, and we now see with much clearer vision the probability, first, that conjugation or its equivalent has primarily the result, as originally interpreted by Bütschli, of offsetting and overcoming the progressive weakening of vitality in infusoria; second, that more or less definite cycles of vigor and depression, ending in natural death unless conjugation or its equivalent supervenes, are characteristic of all pedigreed races of infusoria; third, that physical "immortality" is true of *Paramecium* and other ciliates only in the same sense that it is true of metazoa; fourth and last, that *Paramecium* protoplasm is subject to the same laws of physiological usury that apply to metazoa, and undergoes phenomena which, in metazoa, we call old age, and which, as in metazoa, ends in natural death unless conjugation, or its equivalent parthenogenesis, saves the race.

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[17] Dreitausend und dreihundert Generationen von *Paramecium* u. s. w.," *Biol. Centr.*, Vol. 33, No. 1, 1913, p. 35.

[18] "Ueber Parthenogenesis der Infusorien," etc., *Biol. Centr.*, Vol. 34, No. 9, 1914, p. 577.

THE PHENOMENON OF SELF-STERILITY[1]

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IN both animals and plants in which the two sexes have been combined in the same individual, cases have been found where self-fertilization is practically impossible. This gametic incompatibility has been called self-sterility, although the term is hardly proper as applied to normal functional gametes that may fuse with their complements in the regular manner, provided each member of a pair has been matured in a separate individual.

In plants the phenomenon has been known since the middle of the nineteenth century, in animals a corresponding discovery was made in 1896 by Castle, the species being one of the Ascidians, *Ciona intestinalis*. During the eighteen years that have passed since Castle's discovery, *Ciona* has been studied on a large scale by Morgan (1905), Adkins (Morgan, 1913), and Fuchs (1914). The botanists, however, have lagged somewhat behind; for, in spite of having been acquainted with self-sterility in plants for over half a century, and having found over thirty species where a greater or less degree of self-sterility occurs from which to select material, very few thorough investigations into the physiology of the subject have appeared.

The main facts regarding fertilization in *Ciona intestinalis* are about as follows:

1. Under uniform suitable conditions, individuals vary in degree of self-sterility, it being exceptional to find an animal that is perfectly self-sterile.

2. Self-fertility has never equaled cross-fertility, though the possibility remains that some animals may be self-

[1] Read by title at the thirty-second meeting of the American Society of Naturalists, December 31, 1914.

fertilized as easily as they may be crossed with certain particular individuals.

3. The ease with which the ova of any animal "*A*" may be fertilized by the sperm of other individuals may vary.

Morgan (1913) concluded from his own work and that of Adkins that there were wide differences in the compatibility of ova to different sperm. Fuchs (1914) maintained that 100 per cent. of segmenting eggs can be obtained in every cross if the ova are normal and a sufficiently concentrated sperm suspension is used. It is possible that Fuchs is correct and that varying concentrations of sperm suspension were the cause of Morgan's and Adkins's results, yet the possibility of differences in this regard inherent in the individual is not to be overlooked. It will be seen later that I regard the matter as of great importance to the general subject.

4. A chemical basis for self-sterility is shown in Fuch's experiments by (*a*) the decrease in ease of cross-fertilization after contact of ova with sperm from the same animal, and by (*b*) the difference in ease of self-fertilization after various artificial changes in the chemical equilibrium of the medium surrounding the ova.

From the botanical side various studies on the physiology of self-sterility have appeared since such investigations were initiated by Hildebrand in 1866. At this time it is necessary for us to consider only those of Jost (1907), Correns (1912), and Compton (1913).

Jost was able to show that in self-sterile plants tubes formed from their own pollen were so limited in their development that fertilization did not occur, although the necessary length of pollen tube was easily developed after a cross-fertilization. He saw as the cause of these phenomena the presence of "individueller Stoffe." Pollen was indifferent to "Individualstoff" of the same plant, but was stimulated by that of other plants.

Correns (1912), working with one of the bitter cresses, *Cardamine pratensis,* obtained results to which he gave a simpler interpretation. Starting with two plants, *B* and

G, he crossed them reciprocally and tested 60 of the offspring by pollinating from the parents, on the parents, and *inter se*. The back crosses of $(B \times G)$ or $(G \times B)$ with *B* and with *G* apparently indicated four classes about equal in size with reference to gametic compatibility: (1) plants fertile with both *B* and *G*; (2) plants fertile with *B* but not with *G*; (3) plants fertile with *G* but not with *B*; (4) plants fertile with neither *B* nor *G*.

To these facts Correns gave a Mendelian interpretation by assuming the existence of two factors each of which inhibits the growth of pollen tubes from like gametes. Representing these factors by the letters *B* and *G*, it is clear that types *BB* and *GG* could never be formed. The original plants were supposed to be of classes *Bb* and *Gg*, respectively. When crossed there resulted the four types *BG*, *Bg*, *bG* and *bg*. Plants of types *BG*, *Bg*, and *bG* should be self-sterile, while plants of the type *bg* should be self-fertile. Plants *BG* should be fertile with plants *bg*, plants *Bg* should be fertile with *bG* and *bg*, and plants *bG* should be fertile with *Bg* and *bg*. As a matter of fact Correns's results were not clearly in accord with the theory. Plants of the type *bg* were not self-fertile, and the other classes of matings showed many discrepancies. It is only fair to say, however, that the author recognized some of these difficulties, but believed them to be due to other inhibitors.

In a part of Compton's (1913) work, a still simpler interpretation of self-sterility is offered, at least for a particular case, that of *Reseda odorata*. Darwin's original discovery that both self-sterile and self-fertile races of this plant exist was confirmed and the following results obtained in crossing experiments. Self-sterile plants crossed either with self-sterile or with self-fertile plants gave only self-sterile offspring. Certain self-fertile plants, however, gave only self-sterile offspring when self-pollinated. Other self-fertile plants gave ratios of 3 self-fertile to 1 self-fertile offspring when self-pollinated, and ratios of 1:1 when crossed with pollen from self-sterile

plants. For these reasons he regards self-fertility as a simple Mendelian dominant to self-sterility in the case studied. I believe Compton would draw no such sharp line about self-sterility in general. In fact, he follows Jost in suggesting the presence of a diffusible substance in the tissues of the style and stigma which retards or promotes pollen tube growth after self-pollination or cross-pollination in some manner analogous to the mechanism that promotes animal immunity or susceptibility after infection.

The only alternative general hypothesis has been proposed by Morgan, and this can be discussed more advantageously after the presentation of my own work, of which only an abstract will be given at this time.

In 1909 I made a cross between a small red-flowered *Nicotiana, Nicotiana forgetiana* (Hort.) Sand. and the large white-flowered *Nicotiana* of the garden *Nicotiana alata* Lk. and Otto. var. *grandiflora* Comes. All of the plants of the F_1 generation appeared to be self-sterile. Tests of *Nicotiana forgetiana*[2] have shown these plants also to be self-sterile, but both self-fertile and self-sterile plants of the other parent have been found. From data gathered later, there seems to be no doubt that a self-sterile plant of *Nicotiana alata grandiflora* was used in the actual cross. This conclusion seems reasonable in view of the fact that of over 500 plants of the F_1, F_2, F_3 and F_4 generations tested, not a single self-fertile plant was found.

The plants of the F_1 generation were all vigorous and healthy, and in spite of the fact that they resulted from a species cross which Jeffrey claims always produces large amounts of abnormal pollen, a large number of examinations of pollen from different individuals showed from 90

[2] I thought originally that both of these species (East, 1913) were self-fertile. Seed had been obtained from a carefully bagged inflorescence of each species in 1909. Either the plant of *N. forgetiana* which gave this seed was self-fertile—something that I have never been able to find since that time—or there was an error in manipulation. At any rate, the plants resulting from this seed were all self-sterile.

to 100 per cent. of morphologically perfect pollen grains, a condition about the same as was found in the pure species. To this statement there is one exception. A single plant was found with only about 2 per cent. of good sound pollen.

Several experiments were made in which crossing and selfing was done on a large scale, using plants of the F_2, F_3 and F_4 generations which had segregated markedly in size and were of at least 8 different shades of color. In one of these experiments 20 plants of the F_2 generation coming from 2 crosses of F_1 plants were used. It was planned to make all possible combinations of these plants, 400 in all. This task proved overburdensome, however, and in addition to the self-pollinations but 131 inter-crosses were made with the following results.

1. Each plant was absolutely self-sterile.

2. Leaving out of consideration the plant with shrunken imperfect pollen only two crosses failed. This failure of 1.5 per cent. of the crosses may have been due to improper conditions at the time of the attempts, but as a number of trials were made the possibility remains that there is a small percentage of true cross-sterility.

3. Of the 129 successful inter-crosses, 4 produced capsules with less than 50 per cent. of the ovules fertilized. The remaining crosses produced full capsules. It is barely possible that this result shows a slight variability in ease of cross-fertilization, but I am more inclined to believe that these 4 cases where a low percentage of fertilized ovules were obtained were accidental.

Other crossing experiments of the same kind have corroborated these results. Out of 120 inter-crosses, only 3 failed.

Later, something over 100 inter-crosses were made between 12 plants of an F_3 population resulting from crossing two sister F_2 plants. Six of the attempts at cross-fertilization—3 to 8 trials per plant being made—were failures. These plants as well as others tested were com-

pletely self-sterile, and apparently there was cross-sterility in about 6 per cent. of the possible combinations.

In the F_4 generation, 10 plants resulting from crossing two sisters of the F_3 generation were selected for experiment. Unfortunately, I was able to make only 58 intercrosses, 5 of which, almost 10 per cent., failed.

Back crosses have furnished another line of experiment, though they have not been carried on as systematically as were those of Correns. Nearly 85 back-crosses using plants from the progeny of four combinations which included four individuals as parents, have been made. The plants themselves all proved self-sterile, and in addition 5 of the back crosses failed.

When these experiments were begun I expected to find that the facts would accord with a simple dihybrid Mendelian formula similar to that which Correns later proposed as an interpretation of his results, yet only by considerable stretching and a vivid imagination will Correns's data fit such an hypothesis, and my own data do not fit at all. No self-fertile plants have been produced by any combination, and cross-sterility is a possibility in only from 1.5 to 10 per cent. of the combinations. Furthermore, Correns's idea of inhibitors appears unlikely from some other data I have gathered with the help of Mr. J. B. Park. Ten plants were involved in this experiment. Pairs of plants were provided to furnish series of selfed and crossed flowers. The pistils of these flowers were fixed at regular periods after pollination, stained, sectioned, and the pollen tubes examined. Fertilization not later than the fourth day marked the end point of the crossed series, the dropping of the flowers between the eighth and the eleventh day ended the selfed series. As the flowers on each plant had about the same length pistils, curves of pollen tube development for both crossing and selfing could be constructed. The pollen grains germinated perfectly on stigmas from the same plant, from 1,200 to 2,000 tubes having been counted in sections of single pistils. The difference between the development

of the tubes in the selfed and the crossed styles is wholly one of rate of growth. The tubes in the selfed pistils develop steadily at a rate of about 3 millimeters per twenty-four hours. There is even a slight acceleration of this rate as the tubes progress. If the flowers were of an everlasting nature one could hardly doubt but that the tubes would ultimately reach the ovules, though this would not necessarily mean that fertilization must occur. Since the maximum life of the flower is about 11 days, however, the tubes never traverse over one half of the distance to the ovary. On the other hand, the tubes in the crossed pistils, though starting to grow at the same rate as the others, pass down the style faster and faster, until they reach the ovary in four days or less.

From these facts it seems reasonable to conclude that the secretions in the style offer a stimulus to pollen tubes from other plants rather than an impediment to the development of tubes from the same plant.

The whole question, therefore, becomes a mathematical one, that of satisfying conditions whereby no stimulus is offered to pollen tubes from the same plant, but a positive stimulus is offered to tubes from nearly every other plant.

Morgan has given an answer to this question in a general way. If I understand his position correctly, my own conclusions are not very different from his, but are somewhat more definite. Morgan (1913) states that the results of Adkins and himself on *Ciona intestinalis* can best be understood by the following hypothesis:

> The failure to self-fertilize, which is the main problem, would seem to be due to the similarity in the hereditary factors carried by the eggs and sperm; but in the sperm, at least, reduction division has taken place prior to fertilization, and therefore unless each animal was homozygous (which from the nature of the case cannot be assumed possible) the failure to fertilize can not be due to homozygosity. But both sperm and eggs have developed under the influence of the total or duplex number of hereditary factors; hence they are alike, *i. e.*, their protoplasmic substance has been under the same influences. In this sense, the case is like that of stock that has long been inbred, and has come to have nearly the same hereditary complex. If this similarity decreases

the chances of combination between sperm and eggs we can interpret the results.

I make this quotation to show Morgan's viewpoint. It is for him to say whether the following conclusions are extensions of his own or not.

The tolerably constant rate of growth of pollen tubes in the pistils of selfed flowers, compared with the great acceleration of growth of the tubes from the pollen of other plants as they penetrate nearer and nearer to the ovary, undoubtedly shows the presence of stimulants of great specificity akin to the "Individualstoffe" of Jost. We are wholly ignorant of the nature of these stimulants, but I am inclined towards a hypothesis differing somewhat from his. Experiments by several botanists, which I have been able partially to corroborate, point to a single sugar, probably of the hexose group, as the direct stimulant. The specific "Individualstoffe" I believe to reside in the pollen grains and to be in the nature of enzymes of slightly different character, all of which, except the one produced by the plant itself for the use of its own pollen or by other plants of identical germinal constitutions, can call forth secretion of the sugar that gives the direct stimulus. At least this idea links together logically the fact of the single direct stimulus and the need of "Individualstoffe" to account for the results of the crossing and selfing experiments. But whether or not this be the correct physiological inference, the crossing and selfing experiments call for a hypothesis that will account for no stimulation being offered the tubes from the plant's own pollen, while at the same time great stimulation is given the tubes from the pollen of nearly every other plant.

This is a straight mathematical problem, and it is hardly necessary to say that it is insoluble by a strict Mendelian notation such as Correns sought to give. This is obvious to any one familiar with the basic mathematics of Mendelism. On the other hand, a near Mendelian interpretation satisfies every fact.

Let us assume that different hereditary complexes stim-

ulate pollen tube growth and in all likelihood promote fertilization, and that like hereditary complexes are without such effect. One may then imagine any degree of heterozygosis in a mother plant and therefore any degree of dissimilarity between the gametes it produces, without there being the possibility of a single gamete having anything in its constitution not possessed by the somatic tissues of the mother plant. From the chromosome standpoint of heredity the cells of the mother plant are duplex in their organization; they contain N pairs. The cells of the gametes contain N chromosomes, one coming from each pair of the mother cell; but they are all parts of the mother cell and contain nothing that that cell did not contain. These gametic cells can not reach the ovaries of flowers on the same plant because they can not provoke the secretion of the direct stimulant from the somatic cells of that plant.

All gametes having in their hereditary constitution something different from that of the cells of a mother plant, however, can provoke the proper secretion to stimulate pollen tube growth, reach the ovary before the flower wilts and produce seeds. Such differences would be very numerous in a self-sterile species where cross-fertilization must take place; nevertheless like hereditary complexes in different plants should be found, and this should account for the small percentage of cross-sterility actually obtained. It must be granted that this hypothesis satisfies the facts, but that is not all. It is admittedly a perfectly formal interpretation, but from a mathematical standpoint,—granting the generality of Mendelian inheritance,—it is the only hypothesis possible that can satisfy the facts.

Let us now look into a few of the ramifications of the subject. Examinations of the pistils that have been sectioned after cross-pollination show a considerable variation in the rate of growth of individual pollen tubes, though our curves of growth have been made by taking the average rate of elongation. Is this variation a result

of chance altogether or must we assume a differential rate of growth increasing directly with the constitutional differences existing between the somatic cells and the various gametes? If we assume that any constitutional difference between the two calls forth the secretion of the direct stimulus to growth, chance fertilization by gametes of every type different from that of the mother plant will ensue; if there is a differential rate, selective fertilization will occur. This question can not be decided definitely at present, but two different lines of evidence point toward chance fertilization.

1. Flowers from a single plant pollinated by different males show no decided difference in rate of fertilization.

2. Color differences are transmitted in expected ratios.

Further, it will be recalled that beginning with the F_2 generation, sister plants crossed together have given us our F_3 and F_4 populations, and that these F_3 and F_4 populations apparently have given a constantly increasing percentage of cross-sterility. This is what should be expected under the theory that a small difference in germ plasm constitution is as active as a great difference in causing the active stimulation to pollen tube growth. Breeding sister plants together in succeeding generations causes an automatic increase of homozygosity as is well known. This being a fact, cross-sterility should increase. Such an increase in cross-sterility has been observed in the F_3 and the F_4 generations, but it would not be wise to maintain dogmatically that it is significant.

There are various questions, including the important one of the origin of self-sterility, that can not be discussed at this time. In conclusion, therefore, let us turn once more to the phenomenon of self-sterility in *Ciona intestinalis*. It seems to me that the hypothesis outlined above has few, if any, drawbacks when applied to self-sterility in plants. The question there, as far as we have gone, is one of pollen tube growth, and the theory that the secretion of the direct stimulant can be called forth only by a gamete that differs in its constitution from the somatic

cells between which the pollen tube passes, is logical. If the same theory is to be extended to animals, however, it follows that the external portions of the membranes of the animal egg that have been shown by the wonderful investigations of Loeb and of Lillie to have such important functions, must be functionally zygotic in character. I am aware that this suggestion may be considered pretty radical, but it certainly should be given consideration. I do not like to draw an analogy between the animal egg and a pollen grain, but it may be mentioned that in these structures—surely comparable to the animal egg in the fineness of their membranes and walls—both color and shape are inherited as if they were zygotic in nature.

December 5, 1914.

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THE BLACK-AND-TAN RABBIT AND THE SIGNIFICANCE OF MULTIPLE ALLELOMORPHS

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IT is well known that the European rabbit has undergone great variation, and now exists in a large number of domesticated varieties. Darwin and most other naturalists speak of this as "variation under domestication," implying that domestication has caused the variation. Modern genetic research, however, indicates that domestication has occasioned the preservation rather than the origin of the fundamental variations involved. But to what extent man through selection is able to modify the fundamental variations which nature occasionally produces as sports is still an open question. Evidence is nevertheless accumulating that certain of these fundamental variations may occur in two or more alternative forms, and the question then arises (1) whether these alternative forms have arisen independently by distinct acts of mutation, or (2) whether one has arisen from another by a process of secondary mutation, or (3) whether one may not have been transmuted into another by a more or less gradual process. Toward the testing of these several hypotheses much genetic research is now being directed. The first step to be taken is evidently to ascertain in how many alternative forms the same fundamental variation may occur and how these forms are interrelated. A further step will be the attempt to produce new alternative forms at will. It is our purpose, in this paper, to discuss a newly discovered alternative form (allelomorph) of the gray, or agouti, type of coat found in wild rabbits. It occurs in the variety known as black-and-tan.

This variety appears to have arisen from the wild gray, or agouti, type without the loss of any known genetic factor, but by a modification in one. Simple loss of genetic factors is believed by most students of genetics to have

given rise to black, chocolate and albino varieties of rabbits and other rodents, but a hypothesis of this sort will not fit the present case. No factorial loss can be detected, but only a change in that genetic factor which has been called the *agouti* or gray factor. Under the influence of this factor, what would otherwise be a black variety becomes gray, and what would otherwise be sooty yellow ("tortoise" of the fanciers) becomes clear yellow ("fawn" of the fanciers). This same factor converts chocolate into cinnamon (Punnett, 1912). In every way, accordingly, its influence on the coloration of rabbits is similar to that of the agouti factor in guinea-pigs and mice.

But in mice Cuénot (1909) showed that the agouti factor may assume three distinct forms allelomorphic to each other, the effects of which are seen respectively in gray, light-bellied gray, and in yellow mice. Entire absence of agouti marking from the fur (non-agouti) forms a fourth allelomorph in the series.

In guinea-pigs the agouti factor assumes two alternative conditions, the effects of which are seen in ordinary (light-bellied) agoutis and in agoutis with "ticked" bellies, respectively (Detlefsen, 1914). These two conditions correspond closely in appearance and in order of dominance to the light-bellied gray and the ordinary gray of mice, the former being dominant in both cases. Non-agouti is an allelomorph to both, as in mice.

The peculiarity of the agouti seen in black-and-tan rabbits is that it produces less extensive ticking of the fur than does ordinary agouti. In a typical black-and-tan rabbit the light-colored (yellowish) bands on the hairs, which constitute the "ticking," occur only sparingly on the sides of the body, and not at all on the back or the head. But the under side of the body, including the throat and under surface of the tail, are light (yellowish or whitish) and the back of the neck and inside of the ears bear reddish or yellowish pigment, as in gray rabbits. The typical black-and-tan rabbit of the fanciers has very intense pigmentation which deepens the shade of the "tan" (yellow) found on belly, sides, etc. But this inten-

sity is inherited independently of the agouti factor as crosses with dilute colored varieties of rabbit show. For a cross between black-and-tan and blue produces in F_2 (1) *blue-and-tans* as well as (2) black-and-tans, (3) blacks, and (4) blues. This result is strictly parallel with that obtained by crossing intense gray rabbits with blue ones. In that case there are produced (1) blue gray, (2) intense gray, (3) black, and (4) blue young in F_2. It is evident that in each case a dihybrid cross is made and that the end products are the same in the two series except for the difference in the agouti marking of varieties (1) and (2). The natural conclusion is that black-and-tan contains an alternative form of agouti to that found in gray rabbits. If so, it should be capable everywhere of substitution for gray, wherever the latter occurs throughout the entire series of color varieties, and indeed this appears to be the case.

That the black-and-tan factor, like the ordinary agouti factor, is independent of the extension-restriction pair of allelomorphs is shown by a cross of black-and-tan with sooty yellow (*i. e.*, non-agouti yellow or "tortoise"). F_2 contains (1) black-and-tan, (2) black, (3) yellow ("fawn"), and (4) sooty yellow ("tortoise") young. The first two are varieties with extended pigmentation, and the second two are varieties with *restricted* pigmentation; further, varieties (1) and (3) contain *modified* agouti, but varieties (2) and (4) do not.

If a gray rabbit had been used, instead of a black-and-tan, in making the cross just described, three of the four varieties obtained in F_2 would have been indistinguishable from those enumerated, and the fourth one would merely have been gray instead of black-and-tan. This supports the view that black-and-tan is merely an alternative form of gray.

Further, we have evidence to show that the black-and-tan form of agouti, like the agouti of gray rabbits, is independent of the genetic factors which respectively produce Dutch pattern, English pattern, and angora coat, since we have been able to produce individuals in which black-and-

tan was associated with each one of these Mendelizing characters, as well as others in which it was not associated with them. Finally Haecker (1912) has shown that black-and-tan, like the ordinary form of agouti, is independent of albinism, since when black-and-tans are crossed with Himalayan albinos, not only these two varieties are obtained in the F_2 generation, but also *blacks*. The proportions in which these three varieties were obtained by Haecker approximate the modified dihybrid ratio, 9 black-and-tan: 3 black: 4 Himalayan. One of the two Mendelian pairs concerned is color *vs.* albinism; the other and independent one, black-and-tan *vs.* black.

It is known that if a gray rabbit is used, instead of a black-and-tan one, in a cross with Himalayan albinos, the same 9:3:4 ratio is obtained in F_2, of grays, blacks, and albinos, respectively. The observed results differ, in the two cases, only in the substitution of gray for black-and-tan, which is further evidence that it is only another form of the same genetic factor.

Notwithstanding all this consistent and converging evidence, it is possible that the modified form of agouti seen in black-and-tan is not due to a changed agouti factor itself, but to the modifying action of a factor associated with it which partially inhibits its action. Here we must consider two subordinate possibilities: (*a*) that the supposed modifier is wholly independent of the agouti factor, and (*b*) that it is coupled with the agouti factor. The first possibility is readily disproved; the second one is not so easily disposed of.

(*a*) If black-and-tan were due to the action of an independent modifying factor associated with agouti, a cross of black-and-tan with ordinary black should permit the separation of agouti from its supposed modifier in a considerable part of the F_1 gametes and F_2 zygotes, so that we should expect F_2 to contain gray animals as well as blacks and black-and-tans. But experiments started several years ago at the Bussey Institution show that when black is crossed with black-and-tan no gray offspring are obtained either in F_1 or in F_2, but only black-and-tans in

F_1, and black-and-tans and blacks in F_2. This result shows that black-and-tan is a simple dominant over black.

To establish the allelomorphism of black-and-tan with gray the following experiments may be cited. A black-and-tan rabbit heterozygous for black was crossed with a pure-bred Belgian hare, which variety possesses the genetic color factors of wild rabbits, including the ordinary agouti factor. All the F_1 young were gray, closely resembling Belgian hares, but proved to be genetically of two types. For, when mated with black rabbits, some of them produced gray young and black young, while others (even when mated, as in some cases, with the same black animals) produced gray young and black-and-tan young. This result was quite what was to be expected if gray, black-and-tan and black are mutually allelomorphic conditions. On no other hypothesis which we can suggest was it to be expected. For the black-and-tan parent in the cross was known to be heterozygous for black. It accordingly should form two sorts of gametes, black and black-and-tan respectively, provided that these conditions are allelomorphic to each other. The Belgian hare parent was known to transmit gray in all its gametes. The combinations expected from the cross are therefore of two types, viz.: (1) gray combined with black, and (2) gray combined with black-and-tan. It is well known that gray and black are allelomorphs of each other, the former being dominant. Zygotes of type (1), therefore, should produce gametes of two sorts, gray and black; and when back-crossed with black should produce equal numbers of gray young and black ones but no black-and-tan young. We have tested 12 F_1 gray young from this cross (6 males and 6 females) which are evidently of type (1). Mated with black animals, they have produced 69 gray young, and 65 black ones, but no black-and-tans.

On the other hand 8 F_1 gray rabbits from the cross under discussion have proved to be of type (2), producing gray young and black-and-tan young but no black ones. Together they have produced 44 gray and 51 black-and-tan young, besides 14 other young (two litters) which

were certainly not blacks, since they had light bellies, but which died before attaining the age at which gray can be distinguished from black-and-tan. It is certain that among the 109 young produced by the 8 animals of type (2) not a single one was black.

But if black-and-tan is not an actual allelomorph of gray, black young as well as black-and-tans should have been produced in the foregoing case. For if black-and-tan is not allelomorphic with gray, or is due to an independent inhibitor of gray, then an F_1 gray should produce gametes of *four* sorts, rather than as indicated of *two* sorts; *i. e.*, gametes should arise which transmit *both* gray and black-and-tan, and others which transmit *neither* gray nor black-and-tan. The former sort possibly might not be capable of immediate detection in the back-cross with black, but the latter should be readily discovered since they would necessarily produce black young (neither gray nor black-and-tan). The total absence of black young from the litters produced by type (2) matings therefore indicates strongly that gray and black-and-tan are allelomorphs of each other.

(*b*) An alternative view, however, deserves consideration. If gray and black-and-tan are not actual allelomorphs, it is conceivable that they may each be closely "coupled" with a common structure in the germ cells and so *behave* as allelomorphs under ordinary circumstances, though not being such in reality. Or, what would give the same practical result, gray and black-and-tan might be supposed to contain the same agouti factor, but this might be considered in one as closely coupled with a modifying factor which made its action different. Neither form of this hypothesis is capable of proof or disproof, for which reason alone the hypothesis is unimportant, but its probability grows less the larger the number of records obtained which show no breaking of the supposed coupling. Our cases are not as yet numerous enough to throw much light on this question, but so many cases have already been discovered in which characters assume three or more mutually allelomorphic conditions and in which

no evidence of coupled modifiers has yet been discovered, that the existence of such assumed modifiers seems at present doubtful.

Besides the triple or quadruple series of agouti allelomorphs now known for mice, guinea-pigs and rabbits, at least three other Mendelian factors concerned in the pigmentation of rodents vary discontinuously in this way.

1. Castle (1905) and Punnett (1912) have shown that the Himalayan rabbit possesses a form of albinism allelomorphic with that of ordinary albino rabbits, and that both are allelomorphic to ordinary pigmentation. Guinea-pigs show an even more extended series of albino allelomorphs (Castle, 1914, Wright, unpublished data).

2. Punnett (1912) has discovered in rabbits an alternative form of the "extension" factor, one in the presence of which the agouti factor produces a less amount of ticking than normally. He describes it as a *darkened* extension, *i. e.*, as ordinary extension modified by a coupled darkening factor. This is of course only an alternative form of statement to saying that extension occurs in two forms, for he discovered no cases in which the hypothetical coupling was broken. The three allelomorphs in the case of Punnett's rabbits were accordingly: 1, *ordinary extension;* 2, *darkened extension,* and 3, *restriction.*

3. In still another Mendelian factor affecting the pigmentation of rodents discontinuous variation occurs attended almost certainly by the formation of a series of allelomorphs. Cuénot (1904) stated that white-spotting in mice occurs in a graded series of conditions as regards the amount or extent of the white areas. He found that widely separated stages in the series Mendelize on crossing, *i. e.*, that the segregates fluctuate about modal conditions corresponding roughly with the conditions of spotting found in the respective parents crossed, and he concluded the number of allelomorphs which it would be possible to find in the series to be indefinitely great. Subsequent studies of the subject made by Little (1914) in mice, and by Castle and Phillips (1914) in rats, have not served to simplify the matter, and yet they confirm Cué-

not's general idea that a series of mutually allelomorphic conditions of spotting exists. Unquestionably in rats, "hooded" and "Irish" are such modal conditions of spotting, allelomorphic with each other and with the unspotted or self condition (Doncaster, 1905; MacCurdy and Castle, 1907; Castle and Phillips, 1914). The last-named authors find that independent factorial modifiers probably affect the extent of the spotting and yet that, aside from such modifiers, the spotting factor proper may assume relatively stable allelomorphic conditions which Mendelize when crosses are made between stages sufficiently distinct. The point of especial interest in allelomorphic conditions of spotting is that they are *not* perfectly stable, but are capable of gradual and apparently indefinite modification through the selection of fluctuations either plus or minus. It would be premature to conclude that similar fluctuations (though perhaps less conspicuous ones) do not occur about the modal conditions of other genetic factors which show allelomorphic variation. The black-and-tan form of agouti certainly fluctuates in the amount of ticking found on the sides of the body and the head; doubtless some of this fluctuation may be due to factors genetically distinct from the chief allelomorphic factor concerned, but there is at present no sufficient ground for supposing the chief factor itself to be incapable of fluctuation. Indeed, it seems highly probable, in the light of evidence already obtained, that the present modal condition of the black-and-tan character is one which has been attained only as a result of persistent selection, and that reversed selection will carry it back appreciably nearer to the modal condition seen in gray rabbits. Accordingly, it appears doubtful whether allelomorphs are themselves perfectly and permanently stable. Moreover, the rapid increase of recognized allelomorphs makes us wonder whether their number is limited and definite. Black-and-tan represents, on the whole, an intermediate condition between black and gray. Is it not conceivable that intermediates may yet be discovered be-

tween black-and-tan and black, or between black-and-tan and gray, or even that black-and-tan itself might be displaced to such an intermediate condition by selection of its fluctuations? Here are fruitful fields of inquiry to be cultivated before we conclude with the exponents of "exact" heredity that selection of fluctuations is useless and that only mutations count in evolution.

December 26, 1914.

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DATA ON A PECULIAR MENDELIAN RATIO IN DROSOPHILA AMPELOPHILA

JOSEPH LIFF [1]

A MUTANT with pink eyes was found by Professor T. H. Morgan in the summer of 1910, in one of his culture bottles which contained wild, red-eyed *Drosophila*. He described it as follows:[2]

"The pink eye is more translucent than the red eye, but of about the same general tone. It lacks the dark fleck seen in the red and vermilion eye when the eye is examined with a lens. This black fleck changes its position as the lens travels over the eye. The pink eye, P, is with a little experience easily distinguished from the other colors, especially in newly hatched flies. When the fly gets old the eye turns to a brown color very characteristic of this type of eye."

Pink was found to be recessive to red. The Mendelian expectation in the F_2, viz., three red to one pink, gave the following (Morgan, 1911):

TABLE I

	F_1	F_2		Proportion of Red : Pink
		Red	Pink	
Red ♀ × Pink ♂.....	All red	3,063	169	18 : 1
Pink ♀ × Red ♂.....	All red	1,133	237	5 : 1

The expectation in either case was 3:1, but the numbers realized were 18:1 and 5:1.

In the spring of 1912 I repeated this experiment under the direction of Professor Morgan in order to find whether or not the above ratio would persist. The results have already been published (Morgan, 1912), but a brief summary is here reproduced for reference:

[1] From the Zoological Laboratory of Columbia University.

[2] *Jour. Exp. Zoology*, Vol. 11, No. 4, November, 1911.

TABLE II

Red ♀ × Pink ♂ → in F_2				Pink ♀ × Red ♂ → in F_2			
Bottle	Red	Pink	Proportion of Red: Pink	Bottle	Red	Pink	Proportion of Red : Pink
A....	318	94	3.3 : 1	*a*....	541	124	4.3 : 1
B.....	140	34	4.1 : 1	*b*....	199	63	3.2 : 1
C.....	375	70	5.3 : 1	*c*.....	582	136	4.3 : 1

The above are records of mass cultures. When pairs were used, the fluctuations in ratio were much more marked. The records of 40 pairs gave an almost unbroken series running from 1.8:1 up to 6:1. In seven cases out of the 40 (18 per cent.) the pink flies exceeded the expectation; 3 pairs (7.5 per cent.) gave a 3:1 ratio, while in the remaining 30 pairs (75 per cent.) the pink fell behind. The total number produced by these 40 pairs was 4,056, of which 891 were pink—an average ratio of 3.58:1, about the same as that shown in Table II.

In a second experiment the F_1 hybrids were backcrossed to the pink. The expectation was 1:1. But the records of 15 bottles of mass culture showed fluctuations running from 1:1 up to 2.3:1. The total number counted in these back crosses was 5,527, of which 2,391 were pink, giving an average ratio of 2.31:1. The pink flies fell behind again, and in about the same proportion as in the normal cross.[3]

These remarkable fluctuations were observed at the time the experiments were in progress, and it was suggested that some environmental condition was responsible for the results by either accelerating or retarding[4] the development of the one or of the other variety. The fact that all these experiments were performed at the same time, and the bottles kept side by side in a room in which a nearly constant temperature was maintained throughout the winter, precludes the chance of a factor outside the culture bottles operating here. Attention was there-

[3] For a detailed account of these experiments see Morgan, 1912.

[4] It should be noted here that owing to the danger of overlapping of generations, the bottles were discarded on the tenth day (counting from the day the first F_2 emerged) regardless of the number of unhatched pupæ

fore directed to the condition of the food inside the bottles. An examination seemed to indicate that those in which the food was dry yielded the higher pink ratios. To test this, two of the bottles in which conditions were normal, and in which the F_2 had just begun to emerge, were made "wet" by the addition of a considerable amount of banana juice. But they still showed a similar tendency to yield a relatively higher proportion of pink.

To ascertain more definitely whether or not moisture or dryness affected in any way the development of these flies, a special experiment was arranged in which some flies were bred in "dry" bottles, and some in "wet" bottles. In the first case, the banana was thoroughly dried by means of filter paper which was discarded after it had absorbed all the available moisture, and the banana wrapped in fresh paper; in the second, banana juice was added every second or third day, so that there was throughout the experiment an abundant amount of wet food in the bottles. The effect of this treatment is shown in Tables III and IV:

TABLE III

RECORD OF F_2 OF A CROSS RED BY PINK IN WHICH THE FLIES DEVELOPED IN BOTTLES IN WHICH THE FOOD WAS "DRY"

Pink ♀ × Red ♂						Red ♀ × Pink ♂					
Bottle	Red		Pink		Proportion Red : Pink	Bottle	Red		Pink		Proportion Red : Pink
	♀	♂	♀	♂			♀	♂	♀	♂	
A....	95	86	25	18	4.2 : 1	*a*....	91	109	28	32	3.3 : 1
B.....	73	78	20	21	3.7 : 1	*b*....	76	65	29	25	2.6 : 1
C.....	182	164	39	44	4.3 : 1	*c*.....	43	42	14	15	3.0 : 1
D....	99	89	39	42	2.3 : 1	*d*....	161	117	55	46	2.7 : 1

TABLE IV

RECORD OF F_2 OF A CROSS RED BY PINK IN WHICH THE FLIES DEVELOPED IN BOTTLES IN WHICH THE FOOD WAS "WET" FROM THE BEGINNING

Pink ♀ × Red ♂						Red ♀ × Pink ♂					
Bottle	Red		Pink		Proportion Red : Pink	Bottle	Red		Pink		Proportion Red : Pink
	♀	♂	♀	♂			♀	♂	♀	♂	
A....	91	90	27	38	2.8 : 1	*a*....	82	89	17	17	5.0 : 1
B.....	21	26	12	12	2.0 : 1	*b*....	14	15	8	6	2.0 : 1
C.....	84	83	22	23	3.7 : 1	*c*.....	21	29	2	6	6.0 : 1

TABLE V

RECORD OF 14 ♀'S, 7 RED AND 7 PINK, BRED IN 7 BOTTLES EACH OF WHICH CONTAINED ONE RED AND ONE PINK FERTILIZED FEMALE. THE TABLE SHOWS THEIR RELATIVE PRODUCTIVITY

	Counted on	Bottle *a*				Bottle *b*				Bottle *c*				Bottle *d*				Bottle *e*				Bottle *f*				Bottle *g*			
		Red		Pink		Red		Pink		Red		Pink		Red		Pink		Red		Pink		Red		Pink		Red		Pink	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Hatched from deposits of April 15–20	Ap. 25	7	4	0	0	3	3	0	0	5	3	0	0	20	12	0	0	15	10	0	0	16	7	3	0	♂	...	14	0
	26	9	9	0	0	12	8	5	0	7	7	0	0	3	5	0	0	2	6	0	0	18	8	0	6		...	11	9
	27	2	9	8	4	1	8	12	15	7	3	3	1	1	0	1	0	7	12	1	2	4	4	0	0		...	5	8
	29	5	5	9	8	7	5	5	5	20	5	3	2	...	...	...	...	11	15	1	1	7	8	2	5		...	5	6
	30	7	7	7	6	2	5	6	5	12	9	3	2	...	...	...	...	9	10	2	0	14	10	7	6		...	6	3
	May 1	4	4	12	14	3	4	4	8	14	12	3	9	...	...	...	...	9	14	5	0	18	15	8	10		...	5	8
	2	16	15	12	11	13	11	7	12	1	3	1	1	...	...	...	...	11	8	2	1	6	4	6	4		...	14	6
	3	12	27	11	13	20	18	11	7	....	....	....	1	...	...	...	...	20	12	6	6	0	0	1	2		...	4	9
	4	1	2	2	3	5	12	2	4	....	....	....	....	....	....	....	....	31	18	4	1	....	....	....	....		...	17	17
	6	...	...	...	....	2	0	0	2	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....		...	....	....
Hatched from deposits of April 20–25	Ap. 30	3	3	0	0	15	3	0	0	....	....	....	....	13	1	0	0	4	0	0	0	Killed Ap. 20				0	2	0	0
	May 1	4	2	5	7	15	4	9	3	2	0	0	0	18	7	0	0	6	9	7	12	....	....	....	....	10	9	2	0
	2	0	0	5	5	7	5	2	1	1	4	0	0	8	6	2	0	4	3	6	11	....	....	....	....	4	5	2	4
	3	2	1	4	3	14	11	4	3	2	5	0	0	0	0	0	0	10	8	20	20	....	....	....	....	15	6	9	18
	4	0	2	8	11	8	7	1	2	3	0	1	1	0	0	0	0	1	1	5	7	....	....	....	....	16	13	13	19
	6	...	...	1	6	25	20	2	0	13	6	0	1	0	0	1	0	6	0	8	2	....	....	....	....	20	19	14	20
	8	...	...	2	0	16	17	4	4	7	5	0	0	0	0	1	0	17	15	15	16	....	....	....	....	4	6	13	7
	9	...	...	...	....	13	15	6	5	6	7	0	0	...	...	...	...	4	6	13	7	....	....	....	....	5	2	8	4
	10	...	...	...	....	6	6	4	7	6	2	0	0	...	...	...	...	0	4	0	2	....	....	....	....	2	6	6	4
	11	...	...	...	....	0	1	2	2	0	1	0	0	...	...	...	...	0	2	0	1	....	....	....	....	....	....	....	....
	Total....	72	90	92	101	187	163	86	85	106	72	14	18	63	31	5	0	167	153	95	89	83	56	27	33	76	68	148	142
																						Deduct bottle 1....				....	....	81	66
																										76	68	67	76

It is evident that neither dryness nor moisture has any effect in rendering more favorable the conditions needed for the emergence of either the red or the pink variety. The results show that under unfavorable conditions, large numbers of larvæ and pupæ fail to develop, since the total yield of each and every bottle is far below the normal output; and those which do emerge are but chance survivals.

It was suggested that the reason the pink flies fell behind the expected ratio, was the fact that the mutant was weaker than the wild stock and therefore less likely to come through the larval and pupal stages. If this were the case, they should always fall behind. In many cases, however, they actually exceeded the expectation. Furthermore, they always seemed to be just as vigorous, and to live as long as the wild fly.

The hypothesis which was formed at this stage, and which determined to a large extent the experiments which followed was, that another factor not related to eye-color was at work. Such a factor, if one is assumed to be present, by its independent action might be responsible for the disturbance in ratio. It might, moreover, be present in the wild stock which originally gave rise to the pink, since the wild fly is similarly, though less frequently, affected. It is with the search for such factors that the subsequent experiments will chiefly deal.

Before presenting the data, it will be well to point out some of the possible sources of error which were to a great extent eliminated.

I. The method usually employed in these experiments is as follows: The flies, which are to be cross-bred, are taken out of the culture bottles as soon as they hatch and before they have time to mate. They are then put into a clean, sterilized bottle in mass cultures of about five or more pairs. There they remain till their offspring (F_1) are ready to emerge: 9–10 days in summer and 11–12 or 13 days in winter when the temperature is low. The F_1 flies are placed in fresh bottles for a similar length of

time, and then removed. During the succeeding ten days the F_2 are counted each day as they hatch. The bottle is then discarded for fear of overlapping of generations; for, the F_2 might mate and deposit eggs before removal.

It has, however, been observed that each time a bottle is discarded a considerable number of pupæ and even larvæ remain behind. This being the case, it is possible that the ratio we get does not always represent what actually happens. In order to count the total output, it was decided to transfer the flies to a second bottle on the fifth day. All eggs deposited, during the five days that the parent remained there, would thus have at least fifteen days to develop. It was hoped that, in this way, a more representative ratio would be obtained.

II. It was shown (Tables III and IV) that large numbers of larvæ fail to develop when a bottle becomes too "dry" or too "wet." Considerable care was taken to avoid either of these conditions. If a bottle showed a tendency to dry up, fresh food was immediately added; when it was too wet, the moisture was absorbed by filter paper.

III. The yield of a mass-culture bottle is always relatively small as compared with that of the same number of flies mated in pairs. This would indicate probably greater mortality due to overcrowding. For this reason only pairs were used in the later experiments.

In the first of these experiments pure stocks, both pink and red, were used; for it was believed that if differences existed other than the red-pink distribution, between the two varieties, they would be more emphasized if hybridization had not been effected. The chief purpose, however, was to become familiar with the modes of behavior of the races. The experiment follows:

A number of flies, both pink and red, were isolated within one to six hours after hatching and the sexes kept apart for 3–4 days, after which time they were mated, red to red, pink to pink. Immediately after mating, which took place within five minutes to two hours, the males

were removed. One red and one pink of these females were put into each of seven bottles. In this way the same environmental conditions were secured for the eggs of both. Five days later they were allowed to mate again (not by the same males) and placed in a second set of bottles. The same two females that were together in the first set were also together in the second. There they remained five more days. Counts of the flies that hatched were made from day to day, and the bottles were emptied as long as they continued to yield. The results are given in Table V.

The records of these flies show several interesting and suggestive facts. It will be noted, in the first place, that the length of larval life varies through wide limits. Each bottle contained eggs which were deposited during a period of no more than five days. The hatching periods, however, extended through eleven days in the first set of bottles (April 25–May 6) and twelve days in the second set (April 30–May 12). The flies which emerged first consumed but ten days for development; those which emerged last took at least sixteen days. This phenomenon was more marked where the number produced was larger, suggesting that crowding may retard the development of some individuals.

Of equal interest is the fact that the pink flies invariably began to hatch from 24 to 48 hours later than the red. This was true in the second set of bottles as well as in the first, which proves that it was not due to late maturity of the parents, for, at the time of transfer, they were in the midst of their productive period.

Another point of interest is to be found in the fact that the pink stock was, on the whole, less fertile than the red. In the two bottles '*a*' and '*g*' where the productivity of the two was about equal the red, like the pink, were also low-producers. This is significant, and will be referred to later.

In order to test the above-mentioned facts, the following experiment was performed. F_1 hybrids were mated

in pairs, and transferred during a period of twenty days (May 16–June 4) 13 times, remaining in each bottle from one to two days. Care was taken to count every fly of the F_2 that hatched. The result follows:

TABLE VI

RECORD OF F_2 FLIES WHICH HATCHED FROM EGGS DEPOSITED DURING A PERIOD OF 20 DAYS; DURING WHICH TIME THE PARENTS WERE TRANSFERRED THIRTEEN TIMES

Pink ♀ × Red ♂ → in F_2						Red ♀ × Pink ♂ → in F_2					
Pair	Red		Pink		Proportion Red : Pink	Pair	Red		Pink		Proportion Red : Pink
	♀	♂	♀	♂			♀	♂	♀	♂	
A....	235	209	90	95	2.3 : 1	*a*....	134	118	42	37	3.2 : 1
B.....	220	213	84	75	2.7 : 1	*b*....	105	108	67	64	1.6 : 1
C.....	162	168	65	58	2.7 : 1	*c*.....	189	153	52	53	3.2 : 1
D....	202	119	14	19	9.7 : 1	*d*[5]....	70	58	11	8	6.5 : 1

Here we have a group in which the pink ran sometimes relatively ahead of the red. But the other extreme is also represented in pairs *D* and *d*. The numbers obtained are in each case large enough to be significant.

This experiment was repeated on a larger scale in the fall of 1912. Ten pairs were used for each of these crosses and they were continually transferred as long as they lived. The records follow:

TABLE VII

F_2 RECORD FROM RED-EYED FLIES CROSSED TO PINK-EYED FLIES MATED IN PAIRS. SHOWING THE TOTAL OUTPUT OF EACH F_1 PAIR DURING ITS LIFETIME

PINK ♀ × RED ♂ → IN F_2

Pair	No. of Days Each Pair Lived[6]	No. of Times Transferred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each Pair	Proportion Red : Pink
			♀	♂	♀	♂				
A ..	45	27	604	578	89	81	1,282	170	1,452	7.5 : 1
B ..	19	15	169	145	30	41	314	71	385	4.4 : 1
C...	31	22	320	305	116	94	625	210	835	3.0 : 1
D ..	63	32	658	681	203	221	1,339	424	1,763	3.2 : 1
E ..	20	16	218	218	79	73	436	152	588	2.9 : 1
F...	47	28	476	435	153	144	911	297	1,208	3.0 : 1
G...	22	17	259	248	83	89	507	172	679	2.9+ : 1
H ..	32	22	392	396	152	125	788	277	1,065	2.8+ : 1
I...	63	32	864	805	258	281	1,669	539	2,208	3.1 : 1
J...	25	19	357	358	130	124	715	254	969	2.8 : 1

[5] Lived only eight days.

[6] The length of time a fly lived should not be taken as a criterion for measuring its vigor. In most cases death is accidental.

TABLE VIII

F_2 RECORD FROM RED-EYED FLIES CROSSED TO PINK-EYED FLIES MATED IN PAIRS. SHOWING THE TOTAL OUTPUT OF EACH F_1 PAIR DURING ITS LIFETIME

RED ♀ × PINK ♂ → IN F_2

Pair	No. of Days Each Lived	No. of Times Transferred	Red		Pink		Total Red	Total Pink	Total No. Produced	Proportion Red : Pink
			♀	♂	♀	♂				
a...	28	20	366	294	124	112	660	236	896	2.8 : 1
b...	13	11	163	170	58	49	333	107	440	3.1 : 1
c...	7	5	74	90	22	28	164	50	214	3.2 : 1
d...	14	12	213	195	58	57	408	115	523	3.5+ : 1
e...	22	15	101	106	33	26	207	59	266	3.6 : 1
f...	21	16	215	266	95	78	481	173	654	2.8 : 1
g...	37	24	358	288	113	139	646	252	898	2.6 : 1
h...	45	27	433	444	179	169	877	348	1,225	2.2+ : 1
i...	43	26	546	533	187	185	1,079	372	1,451	2.9 : 1
j...	30	21	298	306	90	83	604	173	777	3.5 : 1

A comparison of Tables VI, VII and VIII suggests the possible presence of high and low pink-producing "strains" in these stocks. To test this, some of the offspring of pair *A* (Table VII) in which the ratio was 7.5 of red to 1 of pink, were inbred for the F_3 in order to see if the same ratio would persist. As there were among the red both homozygous and heterozygous forms, they were each mated to their pink sisters or brothers. This combination would give with the former all red (since red is dominant) and with the latter a ratio of 1:1.

Pair *F* (Table VII) in which the ratio was ideal, 3:1, was chosen for the control, and treated in like manner.

In this as in the preceding experiment the flies were mated in pairs and transferred to fresh bottles every second or third day. A peculiar thing happened. Out of 25 pairs taken from "*A*" only two gave offspring; the remaining 23 pairs were apparently sterile. It could not have been due to bad banana or any other unfavorable condition, for the flies had already been transferred five times and no pupæ were found in any of the other bottles. Furthermore, the 16 pairs of the control which ran parallel to them, and were fed with the same food, did well.

To find out whether these flies were actually sterile, each of the 14 remaining pairs—9 having meanwhile died—were separated and every individual mated to wild red-eyed stock. The sterility of the pink flies, both male and female, was found to be absolute, while all red of both sexes were fertile.

As an additional test, some of the offspring of the last cross were inbred *en masse* in order to extract the pink flies which they would produce, since some of them were heterozygous for eye color.

A small number of pink flies were obtained and mated to their red brother and sisters: each pink female was put in a bottle with 3 or 4 red males, and each pink male with 3 or 4 red females. Out of 19 individuals thus tested, only three were found to be fertile; the remaining 16 were sterile.

These facts seem to indicate that some factor or group of factors which make for sterility were present in the "pink"-containing gamete. The results are the more significant since the hybrid fly, in which this condition prevailed, produced a very low pink ratio. Of the control in which 10 pairs were found to be heterozygous for eye-color with an expectation of 1:1, the following results were obtained:

TABLE IX

RECORD OF THE OFFSPRING OF F_2 FLIES OF A CROSS OF PINK ♀ BY RED ♂ IN WHICH A RATIO WAS 3:1, AS EXPECTED. (See pair *F* of Table VIII)

Red Heterozygous ♀ by (Brother) Pink ♂

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	50	352	348	236	244	700	480	1,180	1.7 : 1
2	27	150	130	170	161	280	231	511	1.2 : 1
3	18	51	85	59	50	136	109	245	1.2+ : 1
4	29	251	245	240	258	496	498	994	1.0 : 1
5	10	147	132	133	112	279	245	524	1.1 : 1

The reciprocal cross gave the following:

Pink Heterozygous ♀ by (Brother) Red ♂

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
I	20	82	89	69	59	171	128	299	1.3+ : 1
II	22	123	124	94	107	247	201	448	1.2 : 1
III	32	223	240	213	247	463	460	920	1.0 : 1
IV	29	215	176	184	163	391	347	738	1.1 : 1
V	40	216	178	101	136	394	237	631	1.7 : 1

It will be seen that all the red flies of the last cross were heterozygous, and should give, on further inbreeding, a ratio of 3 : 1. Four pairs out of five (one being sterile), taken from Pair III of Table IX, gave the following:

TABLE X

RED ♀ × RED ♂ OF PAIR III, TABLE IX

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
a....	40	312	348	43	45	660	88	748	7.5 : 1
b....	23	282	316	80	103	598	183	781	3.3 : 1
c....	40	194	214	60	70	408	130	538	3.1 : 1
d....	14	175	163	47	59	338	106	444	3.2 : 1

Pair *"a"* above gave the same result as pair *"A"* (Table VII)—the ratio in each case being 7.5 : 1. The offspring of the latter were found to contain a high percentage of sterile pink flies, owing to which the attempt then made to test that ratio failed. It was therefore decided to repeat the same experiment with the offspring of this *"a"* pair. As in the former case, the heterozygous red flies were picked out by crossing them to their pink brothers and sisters. The expectation was again 1 : 1. The records follow.

TABLE XI

RECORD OF EIGHT PAIRS, HETEROZYGOUS RED × TO PINK, TAKEN FROM THE OFFSPRING OF PAIR *"a,"* TABLE X

Pink ♀ × Heterozygous Red ♂

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
A...	27	179	176	79	88	355	167	522	2.1+ : 1
B...	12	155	153	83	66	308	149	457	2.0+ : 1
C...	28	163	205	126	159	368	285	653	1.3 : 1
D...	28	243	222	206	219	465	425	890	1.1 : 1

The reciprocal cross gave:

Red Heterozygous ♀ × *Pink* ♂

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
a....	28	282	236	210	260	518	470	988	1.1 : 1
b....	28	296	290	5	5	586	10	596	59.0 : 1
c....	12	144	158	136	152	302	288	590	1.0+ : 1
d....	25	72	113	63	98	185	161	346	1.1+ : 1

The pair with which we started (*F*, Table VII) gave the ideal 3 : 1 ratio; but in each of the three generations which were bred from its offspring (Tables IX, X, XI) there appeared again the same fluctuations which were observed in the preceding experiments, and with even more striking emphasis. Among the offspring of the same pair are found some that give a 3 : 1 ratio and some that give a 7.5 : 1; in a second pair we have some giving 1 : 1, and one giving 59 : 1. The latter especially suggests the presence of a factor that actually inhibits the development of the pink flies, and, moreover, that it is being segregated in a mixed stock.

If the presence of such factor is assumed, we should be able by inbreeding to select stocks in which it is present and in which it is absent. For this reason the experiment recorded above (Table V) was here repeated with some modifications. It will be remembered that in the former, a fertilized red female and a fertilized pink male were placed in each bottle and their offspring, the F_1, counted. In the following experiment, in order to secure segregation, the F_2 were counted. Four virgin pairs, two red and two pink, were taken out of the culture bottles and mated separately. Of the F_1 of each of the four, six pairs were taken out, 24 pairs in all, and bred for the F_2. Unlike the first experiment, the males in this case were allowed to remain with the females throughout the experiment. This insured sufficient sperms for the eggs. Every second day the food was removed, together with the

eggs deposited upon it, and fresh banana supplied. Each two batches of eggs—one deposited by a red female, one by a pink female—were placed together in one bottle so that they might develop side by side and under the same environmental conditions. The result of this experiment is shown in Table XII.

TABLE XII

F_2 OF TWO PINK PAIRS *A*, *B*, AND TWO RED PAIRS *a*, *b*, SHOWING SEGREGATION OF PRODUCTIVITY. EGGS OF *A*I–*a*I, *B*I–*b*I, ETC., WERE DEVELOPED IN THE SAME BOTTLE

	Pink							Red						
	F_1 Pair	No. of Days Each Lived	♀	♂	Total Produced	Ave. Per Day		F_1 Pair	No. of Days Each Lived	♀	♂	Total Produced	Ave. Per Day
Pair *A*	*A* I	44	450	446	896	20.40	Pair *a*	*a* I	20	233	219	452	22.60
	A II	24	169	224	393	16.36		*a* II	22	9	8	17	0.77
	A III	28	61	67	128	4.57		*a* III	25	63	64	127	5.10
	A IV	30	221	233	454	15.13		*a* IV	31	272	232	504	16.26
	A V	36	331	345	676	18.77		*a* V	30	179	171	350	11.66
	A VI	21	37	33	70	3.33		*a* VI	22	152	138	390	17.73
Pair *B*	*B* I	29	199	217	416	14.34	Pair *b*	*b* I	44	122	104	226	5.10
	B II	24	93	93	186	7.75		*b* II	28	103	77	180	6.43
	B III	35	31	47	78	2.23		*b* III	44	302	256	558	12.70
	B IV	30	15	13	28	0.93		*b* IV	35	22	34	56	1.60
	B V	28	11	10	21	0.75		*b* V	31	302	293	595	19.20
	B VI	18	10	8	18	1.00		*b* VI	44	154	139	293	6.65

Segregation with respect to productivity is here evident. Whether the low fertility[7] seen in so large a proportion of these flies was due to an actually low egg-production, or whether it was due to something which prohibited development or to some defect in the germ cell owing to which fertilization could not be effected, is not known. That one of the latter possibilities is likely to be realized here can be inferred from the work of Dr. R. R. Hyde in this laboratory. He counted the eggs of hundreds of individuals, and compared them with the number of flies which emerged from them. According to

[7] The term "fertility" is used here, as defined by Hyde, to indicate the number of eggs that complete development and give rise to mature flies. (See Hyde, *Jour. Exp. Zool.*, August, 1914, p. 185.)

his observations, only about 75 per cent. of the eggs of the wild fly ever reach maturity, and in some of the mutations no more than 25 per cent. of the eggs develop.

Another point of interest brought out in the last experiment is the fact that the wild, red-eyed fly behaves in exactly the same manner as the mutant pink fly. This may be the reason for the observed shifting of the ratio sometimes in favor of the one variety, sometimes in favor of the other. It shows furthermore that it was not the pink as such that caused the disturbance. The red also might be similarly disturbed and perhaps by the same agent or by another agent that affected the productivity in the same way.

If the abnormally low number produced by some of the pairs of Table XII be due to the inability of a large number of their eggs to develop, and if we assume this character to be transmissible, it must reappear in the F_2 of a cross in which one of the parents possessed this factor, *i. e.*, a large number of individuals, one quarter of the output, should fail to develop. This would be in accordance with Mendelian principles. A number of crosses were therefore made in various combinations with the individuals taken from Table XII. The results follow:

TABLE XIII

F_2 OF 16 PAIRS OF A CROSS RED BY PINK IN WHICH THE PARENTS CAME FROM *A*I, *a*I, TABLE XII, THE AVERAGE DAILY PRODUCTIVITY OF WHICH WAS 22 AND 20, RESPECTIVELY

A. Pink ♀ (*Productivity 20 Per Day*) × *Red* ♂ (*Prod. 22 Per Day*)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	17	301	294	110	104	595	214	809	2.80 : 1
2	32	484	457	160	147	941	307	1,248	3.01 : 1
3	32	504	570	181	189	1,074	370	1,444	2.90 : 1
4	32	559	548	205	200	1,107	405	1,512	2.73 : 1
5	8	125	126	40	35	251	75	326	3.35 : 1
6	18	261	243	98	89	504	187	691	2.70 : 1
7	21	391	449	155	142	840	297	1,137	2.86 : 1
8	32	673	700	203	236	1,373	439	1,812	3.10 : 1
Total number produced by 8 pairs						6,685	2,294	8,979	

Average proportion, 2.91 : 1

B. Red ♀ (Productivity 22 Per Day) × Pink ♂ (Prod. 20 Per Day)

Pair	No. of Days Each was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
I	30	415	395	135	127	810	252	1,062	3.21 : 1
II	14	132	121	41	73	253	114	367	2.22 : 1
III	24	317	337	97	94	654	191	845	3.42 : 1
IV	30	484	378	119	120	862	239	1,101	3.60 : 1
V	24	392	418	96	140	810	236	1,046	3.42 : 1
VI	30	656	592	198	200	1,248	398	1,646	3.14 : 1
VII	30	566	647	182	209	1,213	391	1,504	3.10 : 1
VIII	24	438	451	132	153	889	285	1,174	3.10 : 1
Total produced by 8 pairs.....						6,739	2,106	8,845	

Average proportion, 3.24 : 1

If the proportion of red to pink, realized in the F_2, depends upon the relative fertility of the two parents which form the cross, we should get in this case, where the parents were supposedly equally fertile, the ideal 3:1 ratio. The records, however, show considerable fluctuations. Nevertheless, these results are perfectly in accord with our hypothesis. Looking back to Table XII, which furnished the parents of this cross, the explanation is obvious. The averages per day for *A*I–*A*VI were 20, 16, 4, 15, 18 and 3, respectively. Similarly, *a*I–*a*VI gave 22, 0.7, 5, 10, 16, 11 and 17, respectively. It is therefore reasonable to assume that among the offspring of *A*I (productivity 20) and *a*I (productivity 22) individuals should be found which would repeat the series. Fluctuation is, therefore, to be expected. The average of many such pairs, however, should be 3:1. The proportion obtained was 2.91:1 in one case; 3.24:1 in the other, or a general average of 3.08:1.

It should also be noted here that in this as well as in the subsequent experiments, wherever eight pairs are recorded, they are not the offspring of one, but of two distinct crossings of one pair each which were made at the same time; that pairs 1–4, 5–8; I–IV, V–VIII, respectively, were brothers and sisters. More than one line is thus represented in each case. With these facts in mind, we may pass on to the remaining experiments.

TABLE XIV

F_1 OF 12 PAIRS OF A CROSS RED BY PINK IN WHICH THE PARENTS WERE *A*I AND *b*II (TABLE XII) THE AVERAGE DAILY PRODUCTIVITY OF WHICH WAS 20 AND 6, RESPECTIVELY

A. Pink ♀ (*Productivity 20 Per Day*) × *Red* ♂ (*Productivity 6*)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	32	355	331	117	95	686	212	898	3.23 : 1
2	28	340	373	126	132	713	258	971	2.76 : 1
3	19	330	288	120	124	618	244	862	2.52 : 1
4	32	544	562	188	188	1,106	376	1,482	2.94 : 1
5	23	574	578	203	214	1,152	417	1,569	2.76 : 1
6	14	196	194	66	59	390	125	515	3.12 : 1
7	32	648	603	222	246	1,251	468	1,719	2.67 : 1
8	32	429	374	141	144	803	285	1,088	2.81 : 1
Total number produced by 8 pairs.....						6,719	2,385	9,104	

Average proportion, 2.84 : 1

B. Red ♀ (*Productivity 6 Per Day*) × *Pink* ♂ (*Productivity 20*)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
I	28	488	482	176	177	970	353	1,323	2.74 : 1
II	6	83	78	21	30	161	51	212	3.15 : 1
III	28	463	465	164	153	928	317	1,245	2.93 : 1
IV	21	375	383	114	116	758	230	988	3.20 : 1
Total number produced by 4 years.....						2,817	951	3,768	

Average proportion, 2.96 : 1

In most of these pairs the pink slightly exceeded the 3:1 expectation. In the few in which they fell behind, the red (if we assume fertility to be the cause) might have been of a higher fertility than the pink, as has been explained. As a group, however, they give a proportion somewhat below 3:1.

In the next cross, the red fly was the more fertile. The results are given in the following table:

TABLE XV

F_2 OF A CROSS RED BY PINK IN WHICH THE PARENTS WERE *B*IV AND *a*I (TABLE XII) THE AVERAGE DAILY PRODUCTIVITY OF WHICH WAS 1 AND 22, RESPECTIVELY

A. Pink ♀ (*Productivity 1 Per Day*) × *Red* ♂ (*Productivity 22*)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	22	398	405	120	105	803	225	1,028	3.52 : 1
2	28	512	477	107	125	989	232	1,221	4.26 : 1
3	22	420	389	104	135	809	239	1,048	3.34 : 1
4	18	340	300	86	106	640	192	832	3.33 : 1
5	19	420	428	129	134	852	263	1,115	3.24 : 1
6	19	372	396	100	104	768	204	972	3.76 : 1
7	17	198	220	58	83	418	141	559	3.00 : 1
8	17	174	212	62	62	386	124	510	3.10 : 1
Total number produced by 8 pairs.....						5,665	1,620	7,285	

Average proportion, 3.50 : 1

B. Red ♀ (*Productivity 22 Per Day*) × *Pink* ♂ (*Productivity 1*)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
I	31	264	262	101	87	526	188	714	2.80 : 1
II	27	353	350	80	85	703	165	868	4.26 : 1
III	31	430	403	97	112	833	209	1,042	4.00 : 1
IV	31	427	420	111	128	847	239	1,086	3.54 : 1
V	28	486	471	122	147	957	269	1,226	3.60 : 1
VI	28	573	568	139	154	1,141	293	1,434	3.90 : 1
VII	28	505	505	116	127	1,010	243	1,253	4.15 : 1
VIII	24	502	501	134	140	1,003	274	1,277	3.66 : 1
Total produced by 8 pairs						7,020	1,880	8,900	

Average proportion, 3.73 : 1

Of the 16 pairs of this cross only one gave less than 3 : 1. In the remaining 15, the proportion was, in each case, considerably higher than 3 : 1. It will be noted that of all 16 pairs that one was the least fertile. This would indicate, on the hypothesis suggested, that the gamete containing the "red" factor did not have relatively as high a potential of fertility as did the parent which produced it.

A comparison of Tables XIV and XV shows that we have two distinct groups: one in which the extracted pink exceed the expectation, and one in which they fall behind

the expectation. Yet the method employed in each case was the same; the history of each is the same. The only difference is to be found in the fact that in the one case the pink came from a more fertile parent; in the other, the red.

The offspring of pairs "7" (Table XIV) and "2" (Table XV) in which the ratios were 2.67:1 and 4.26:1, respectively, were inbred for the F_3. Fifteen pairs were taken from each, but as there were among the red both homozygous and heterozygous flies, only eight gave pink in each case. The results follow:

TABLE XVI

RECORD OF 8 PAIRS HETEROZYGOUS RED-EYED F_2 OF PAIR "7" (TABLE XIV) IN WHICH THE RATIO WAS 2.67:1

Pair	No. of Days	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	14	270	279	80	78	549	158	707	3.47 : 1
2	14	159	167	58	42	326	100	426	3.26 : 1
3	14	5	7	2	1	12	3	15	4.00 : 1
4	14	135	143	46	49	278	95	373	2.93 : 1
5	14	183	157	49	50	340	99	439	3.23 : 1
6	14	172	154	45	42	326	87	413	3.74 : 1
7	14	142	137	43	49	279	92	371	3.00 : 1
8	14	57	46	12	16	103	28	131	3.67 : 1

TABLE XVII

RECORD OF 8 PAIRS HETEROZYGOUS RED-EYED F_2 OF PAIR "2" (TABLE XV) IN WHICH THE RATIO OF RED TO PINK WAS 4.26:1

Pair	No. of Days	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	14	295	308	100	97	603	197	800	3.06 : 1
2	14	392	326	97	105	718	202	920	3.55 : 1
3	14	281	288	95	86	569	181	750	3.14 : 1
4	14	110	104	32	33	214	65	279	3.31 : 1
5	14	167	170	70	59	337	129	466	2.61 : 1
6	14	314	277	82	86	591	168	759	3.52 : 1
7	14	160	169	32	41	329	73	402	4.50 : 1
8	14	133	146	49	41	279	90	369	3.10 : 1

These results are significant, in that they show that the original ratios, which their parents gave, were lost.

The fact that it has been found possible by proper manipulation to get a group in which the ratio fluctuated in one direction only, even if it was not as marked as was hoped it would be, indicates that the disturbance is due to an *internal,* and not to an external, cause. This was further emphasized by the distinct tendency for segregation, as was to be expected if there were some heterozygous individuals. It was also suggested in another way. No matter how short-lived or how long-lived a pair was; whether it was transferred once, twice, or even twenty times, the ratio of red to pink did not vary throughout its life when the yields of the several bottles were compared with one another.

TABLE XVIII

F_2 OF FOUR PAIRS (*A, B, C* AND *D*) RED ♀ × PINK ♂ IN WHICH THE MALES WERE CROSSED EACH TO SEVERAL OF HIS OWN DAUGHTERS

Expectation 1:1

Pink ♂	No. of ♀	Total Red	Total Pink	Proportion Red : Pink
A ×	1	97	45	2.15 : 1
	2	105	71	1.46 : 1
	3	231	161	1.43 : 1
	4	171	122	1.40 : 1
	5	211	194	1.10 : 1
B ×	1	110	80	1.38 : 1
	2	263	251	1.00 : 1
	3	242	198	1.28 : 1
	4	183	143	1.28 : 1
	5	231	189	1.22 : 1
	6	162	102	1.62 : 1
C ×	1	201	113	1.78 : 1
	2	257	116	2.22 : 1
	3	255	206	1.27 : 1
	4	206	153	1.36 : 1
	5	243	197	1.23 : 1
	6	125	98	1.27 : 1
D ×	1	179	135	1.30 : 1
	2	217	121	1.80 : 1
	3	107	74	1.44 : 1

TABLE XIX

F_2 OF PAIRS *A, B, C* AND *D* OF TABLE XVIII

Expectation 3:1

F_1 of Pair	No. of Pair	Total Red	Total Pink	Proportion Red : Pink
A	1	153	41	3.73 : 1
	2	158	55	3.00 : 1
	3	96	21	4.60 : 1
B	1	140	43	3.27 : 1
	2	215	72	3.00 : 1
	3	136	71	1.92 : 1
C	1	50	21	2.38 : 1
	2	125	43	2.90 : 1
	3	122	49	2.49 : 1
	4	193	67	2.88 : 1
D	1	26	6	4.33 : 1
	2	112	34	3.30 : 1
	3	194	74	2.62 : 1

The proportion of red to pink was found to bear a direct relation to the relative "fertility" of the parents which produced the hybrid. This suggests a causal relation between the two.

In dealing with "fertility" the difficulty that one encounters is, that the offspring of any pair may, with respect to this character, differ from either parent, and also differ amongst themselves, forming a graded series running from the most to the least fertile. An individual taken from such a population is an indefinite quantity and will often defeat the purpose of the experiment. In order to simplify this as far as possible, the following experiment was planned:

Four red-eyed, virgin females were each mated to a pink male. Each male was again crossed to several of his own daughters. The records are given in Table XVIII. As a control a number of F_1 pairs were bred in each case. The records are given in Table XIX.

A graphic representation of all pairs recorded in Tables VI–XIX, except for the several very unusual ratios, is given in Figs. 1 and 2.

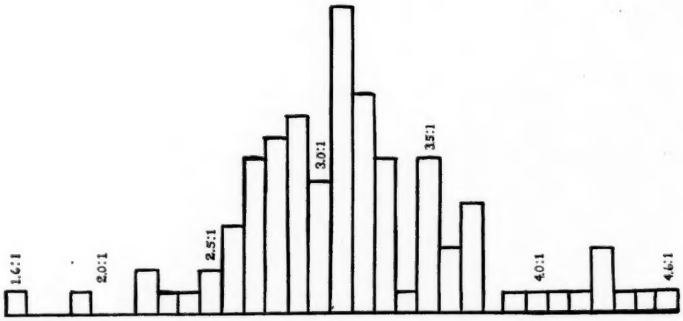

Fig. 1

Fig. 1 contains 99 pairs in each of which the expected ratio was 3:1, with a total population of 82,607. They are distributed as follows:

No. of Pairs		Ratios of Red : Pink
1	gave	1.6 : 1
0	gave	1.7 : 1
0	gave	1.8 : 1
1	gave	1.9 : 1
0	gave	2.0 : 1
0	gave	2.1 : 1
2	gave	2.2 : 1
1	gave	2.3 : 1
1	gave	2.4 : 1
2	gave	2.5 : 1
4	gave	2.6 : 1
7	gave	2.7 : 1
8	gave	2.8 : 1
9	gave	2.9 : 1
6	gave	3.0 : 1
14	gave	3.1 : 1
10	gave	3.2 : 1
7	gave	3.3 : 1
1	gave	3.4 : 1
7	gave	3.5 : 1
3	gave	3.6 : 1
5	gave	3.7 : 1
0	gave	3.8 : 1
1	gave	3.9 : 1
1	gave	4.0 : 1
1	gave	4.1 : 1
1	gave	4.2 : 1
3	gave	4.3 : 1
1	gave	4.4 : 1
1	gave	4.5 : 1
1	gave	4.6 : 1
Total.. 99		

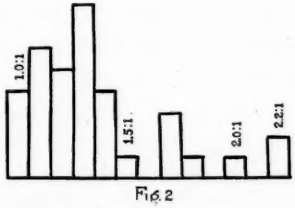

Fig. 2

Fig. 2 contains 37 pairs of back-crosses; expected ratio 1:1; total population 17,008. They are distributed as follows:

No. of Pairs		Ratios of Red : Pink[8]
4	gave	1.0 : 1
6	gave	1.1 : 1
5	gave	1.2 : 1
8	gave	1.3 : 1
4	gave	1.4 : 1
1	gave	1.5 : 1
1	gave	1.6 : 1
3	gave	1.7 : 1
1	gave	1.8 : 1
0	gave	1.9 : 1
1	gave	2.0 : 1
1	gave	2.1 : 1
2	gave	2.2 : 1
Total.. 37		

To these should be added:

1 pair which gave 9.7 : 1, Table V.
1 pair which gave 6.5 : 1, Table VI
1 pair which gave 7.5 : 1, Table VII
1 pair which gave 7.5 : 1, Table X
1 pair which gave 59.0 : 1, Table XI

Except for the several detached pairs at the extreme limits, Fig. 1 shows a normal curve. A disturbance of 0.5 in either direction (less than 10 per cent.) is quite within the limits of experimental accuracy. The larger disturbances, ratios of 6 or 7 : 1, and also the first results reported by Morgan ('11 and '12) are yet to be explained. These are too large to be attributed to experimental error.

The data presented in the foregoing pages show that there has been a marked improvement in the ratio of pink to red since 1911. In one case only (1913) was the disturbance greater than those of Morgan (59 : 1). The remaining very marked disturbances were between 6 and 10 : 1. And these appeared so infrequently that in mass-cultures their presence would hardly have been felt.

A corresponding improvement has also been observed in the fertility of the pink-eyed race between 1912 and 1913. This is seen on comparing Tables V and XII. In the first, the fertility of the pink was much lower than that of the red; in the second (about one year later), it was as high.

Hyde ('14) showed that in some races of *Drosophila*

[8] None gave a ratio of less than one red to one pink.

ampelophila, the number of eggs failing to reach maturity is between 25 per cent. and 75 per cent. of the total output; and concluded that this peculiarity probably behaves as a Mendelian recessive factor. More recently, Morgan ('14) describes recessive lethal factors in *Drosophila,* which he defines, "as any factor that brings about the death of the individual in which it occurs, provided that its effect is not counteracted by the action of its normal allelomorph."

In the light of this evidence, the following conclusions suggest themselves:

1. The original pink-eye mutant was heterozygous for some non-sex-linked factor which, in the homozygous state, acts like Morgan's lethal. This factor was, in the course of time, to a large extent eliminated, as is to be expected if the individuals homozygous for it are more likely to die. The chance of such homozygous forms appearing again, has thereby been much reduced. This is borne out by, and also explains, the improvement in the pink race.

2. A similar recessive, though not necessarily the same factor, might also be present in some individuals of the wild, red-eyed stock. Hyde's work mentioned above gives weight to this assumption—which is not at all an unreasonable assumption in a species as unstable as this, judging by the vast number of mutations reported. For this reason, the red sometimes fall behind the expected ratio.

3. The mode of action of these lethals shows that they are linked to the "pink" factor or to its normal "red" allelomorph. This will be clear from the following analysis:

Of the flies recorded in Fig. 2, one parent was *RP* (with gametes *R* and *P*); the other was *PP* (with gametes *P* and *P*). The zygotes resulting from these gametes almost invariably give fewer *PP*'s than *RP*'s. In other words, the homozygous forms run behind the heterozygous forms. The relation between these two classes may also be supposed to hold in the F_2 cross (Fig. 1). Here, however, the reds (*RR* and *RP*) run relatively less often ahead of

PP. This must be due to a deficiency in the homozygous *RR* flies. In other words, the results taken all together (Figs. 1 and 2) show that the disturbance is brought about by factors (in the third chromosome) which in the homozygous state act as lethals or perhaps as semi-lethals. Random introduction of one or two or no lethals may be assumed, as follows:

(*A*) If the lethal is introduced by the "pink-bearing" chromosome, the homozygous pink will be depressed in the F_2.

(*B*) If introduced by the "red-bearing" chromosome, the homozygous red will be depressed in the F_2.

(*C*) If two lethals, both of which are identical, are introduced at the same time, one by the red and one by the pink, all classes will be equally depressed,[9] and the results as far as concerns the F_2 ratio will be the same as if there were no lethals present, *i. e.*, the 3:1 ratio will be realized.

(*D*) If two lethals that are different are introduced at the same time, one by the red and one by the pink, both the homozygous classes (*RR* and *PP*) will be depressed, but not the *RP*. There would be somewhat fewer pinks than expected in the F_2.

I wish to acknowledge my indebtedness to Professor T. H. Morgan, whose kind attention and suggestions both throughout the foregoing experiments and in the preparation of the present report, were invaluable. I also wish to express my appreciation to Mr. H. J. Muller to whom I owe some suggestions concerning the interpretation of the results.

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[9] "Crossing-over" is ignored as the character of the results is not changed thereby.

SHORTER ARTICLES AND DISCUSSION

SELECTION, SUGAR-BEETS AND THRIPS

A DISCOVERY of great importance to students of genetics has recently been made by one of the plant-breeders[1] of the U. S. Department of Agriculture, viz., that beets are regularly cross-pollinated and that an important agent in the process is a minute inconspicuous insect, so small that it readily passes "through the meshes of fine silk chiffon."

To understand fully the theoretical importance of this discovery one need only recall the large attention given to the sugar-beet in recent adverse criticisms of the selection-theory. De Vries in his "Mutationstheorie," p. 72, cites the case of the sugar-beet as showing the most systematic, refined and elaborate selection known for any cultivated plant, and yet as being without any permanent effect in raising the sugar content of the beet. For, although the average sugar content of the beet has by systematic selection been practically doubled in the last 60 years, De Vries holds the improved racial condition to be unstable and thinks that the improved race would within a few generations revert to its old level of sugar-content if the selection were discontinued. His reason for thinking so is the familiar fact that the offspring of the *best* selected beets are on the average not quite so good as their selected mother-beets, but show a tendency to regress downward toward the old level of sugar content. It should be pointed out, however, that in reality regression is not toward the *original* average of 7 or 8 per cent. sugar-content, but toward an average twice as high as this. For De Vries's variation polygon (*l. c.*, Fig. 22) for the sugar content of 40,000 beets shows a nearly symmetrical probability curve about a mode at 15.5 per cent. It is to be supposed therefore that regression would occur *toward this* condition from both the upper and the lower halves of the frequency polygon, rather than toward the old average condition of 7–8 per cent., which, according to the data of DeVries, is now rarely if ever seen in the improved race. To have doubled the average sugar-content of the beet is certainly something of an achievement for selection; the form of

[1] Shaw, Harry B., "Thrips as Pollinators of Beet Flowers," Bull. No. 104, U. S. Dept. Agr., July 10, 1914.

the variation polygon indicates that the change is permanent, so far as ordinary racial characters have permanency.

But why, it may be asked, has selection not achieved *more* in this case? Why should the descendants of, say, a 25 per cent. beet not score better than this? There are probably several reasons why. (1) Physiological reasons probably offer obstacles. A beet can not be formed which is *all* sugar. There has to be present in the beet a machinery for *manufacturing* the sugar. Perhaps 25 per cent. is an impossibly high average for a race of beets. (2) Perhaps the exceptional 25-per-cent. beet owes its extra sweetness in part to environmental causes which are not permanent. In that case the extra sweetness is "somatic rather than germinal," as we should say in the case of an animal.

(3) Finally the discovery that beets are never self-fertilized, but in every generation are cross fertilized, explains why improvement of the beet through selection is so slow and tedious a process. What progress could the animal breeder expect to make if he were able to select only the dams, but never the sires, for his flocks? This is the condition which confronts the plant breeder in attempting to improve the sugar beet. The animal breeder is often chided with the small numbers which his experiments yield as compared with the enormous numbers which an experiment with plants may produce, but the animal breeder has at least this satisfaction that when the animals are securely penned there need be no uncertainty about pedigrees.

The careful observations of Shaw show that thrips, so common in the blossoms of plants and yet so minute as easily to escape notice and to penetrate within silk nets and under paper bags, may be a cause of unsuspected cross-pollination and unaccountable "mutation" in the breeding of cereals and other plants.

W. E. CASTLE

BUSSEY INSTITUTION,
October 24, 1914

A NOTE ON MULTIPLE ALLELOMORPHS IN MICE

PROFESSOR T. H. MORGAN has recently published in this journal the results of some of his experiments on color inheritance in mice. In this paper he offers material which he considers "evidence establishing" a series of multiple allelomorphs. His series consist at present of four forms, "yellow, gray white-belly, gray

gray-belly and black." The essential point of his conclusion is that no more than two of these conditions can be transmitted by any one animal.

The fact that Cuénot in his series of classic papers on color inheritance in mice (1902–1911) recognizes these same four types as forming a group of allelomorphs is not mentioned by Morgan, whose paper, without knowledge of Cuénot's work, might well be taken to contain "the evidence establishing this series of allelomorphs" as he himself considers that it does. Since Morgan appears to have overlooked Cuénot's work with these forms, it may be interesting to give a brief statement of Cuénot's results.

As early as 1903 Cuénot recognized that albinos, potentially yellows, when crossed with black gave besides yellow offspring *either* black or agouti young, but not both. This is, of course, evidence that yellow, agouti and black are all allelomorphic to one another. In 1904 he gives formulæ (p. 46) showing that he considers this to be the case. At the same time he gives the ratios produced by crossing an albino potentially a *heterozygous gray* (agouti) with a yellow carrying black, but no agouti, and albinism. For present purposes the albinism in the cross is negligible. Cuénot recognized that the ratio expected from this cross was 2 yellow, 1 black and 1 agouti (gray). He obtained 34 yellow, 20 black and 16 agouti; the calculated numbers being 38:19:19. Sturtevant (1912) in discussing the allelomorphism or coupling of black, agouti and yellow in mice has also overlooked Cuénot's results, for in mentioning the cross of a heterozygous agouti with a yellow carrying black, he states "apparently Morgan is the only one who has reported such a cross. He obtained 4 yellows, 5 agoutis and 1 black."

To return to Cuénot's work; in 1907 he made a report on the hereditary behavior of the white bellied agouti variety (gris à ventre blanc) which he considers allelomorphic to yellow, agouti and black. On page 10 in speaking of *determinants* he says: "Il y en a le même nombre dans les races unicolores et dans la race grise; ces races diffèrent, non pas par la quantité de leurs déterminants mais par la qualité." This is essentially the idea underlying multiple allelomorphism. Later in the same paper he says of G, the agouti determinant ". . . il présent un grand nombre de mutations: G′, N et J." (G′ = white bellied gray; N = black and J = yellow.) On page 13 he tabulates the varieties, *in order of their dominance,* yellow, white-bellied agouti,

agouti and black. Morgan reached the same order of dominance in 1911 and has recently (1914) recorded them, beginning with black, as follows:

b = black,
B^G = gray gray-belly,
B^w = gray white-belly,
B^y = yellow.

In 1908 Morgan published certain facts concerning the inheritance of the white-bellied gray pattern. Cuénot at once (1908) publicly called Morgan's attention to the similarity of their material and added facts which showed that he had already investigated the inheritance of this same pattern in 1907. Morgan later acknowledged its similarity.

In 1911 Cuénot states plainly (p. 47): "Les souris jaunes sont characterisées par un déterminant J, allélomorphe a G, G' et N, at qui les domine tous dans les croisements . . . il n'y à que les zygotes renfermant J dominant un autre déterminant allélomorphe (G, G' ou N) qui peuvent évolouer."

Morgan's 1914 paper adds several detailed matings and records the testing of yellows of both sexes. However, in most respects, his work corroborates the pioneer experiments of Cuénot and does so in such detail that he falls into the same error as did Cuénot in considering "black" as a necessary member of the allelomorphic series. This is obviously incorrect for the whole series of allelomorphs exists equally well in forms *utterly lacking the ability to produce black pigment* as some of Morgan's experiments showed. The true series of allelomorphs is yellow, white bellied agouti, gray-bellied agouti and *non agouti* (not black).

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BUSSEY INSTITUTION,
October 19, 1914

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ON THE TIME OF SEGREGATION OF GENETIC FACTORS IN PLANTS

IN *Œnothera lamarckiana,* Geerts (3) has observed that two microspores of each tetrad abort. From the results of reciprocal crosses, De Vries (9) concluded that there was a segregation of genetic factors between the aborted and unaborted pollen-grains. In my crosses of *Stizolobium* species (2), half of the pollen-grains abort in a random manner in the anthers of the F_1 hybrids; and I can only explain the results of the breeding work on the hypothesis that there is a segregation between the four microspores of each tetrad. Hence I conclude that the segregation does not take place before the cell-divisions which form the pollen-mother-cells, but takes place in the divisions which form the microspores. In other words, segregation occurs here, not among the cells of the diploid generation, but at the moment of formation of the individuals of the haploid generation.

In the ovules of *Stizolobium* crosses I have shown that there is a random segregation of aborted and normal embryo-sacs; and this agrees with the observations of Geerts on the functional megaspores of *O. lamarckiana.* If somatic segregation occurred, there would be a segregation of whole ovaries or parts of ovaries with ovules all aborted or all normal, causing a distribution which would differ markedly from the binomial distribution demanded by a random segregation according to the law of chance. I have shown that with lots of n ovules each, the distribution of the aborted and normal ovules corresponds to the binomial $(1+1)^n$. Hence segregation can not have taken place before the formation of the nucellus of the ovule.

In many species and varieties of *Citrus,* as Strasburger (7)

and Osawa (4) have proved (and as I can confirm), embryos are formed from the tissue of the nucellus adjacent to the embryo-sac. I have also shown (1), as Strasburger suspected, that a similar mode of formation prevails in certain varieties of *Mangifera indica.* In F_1 hybrids between certain *Citrus* species (8), these adventive embryos do not show segregation; and the adventive embryos of a mango variety give plants nearly constant to that variety. Hence segregation had not taken place when the cells surrounding the megaspore-mother-cell were formed.[1] The same conclusion follows on the work of Ostenfeld (5) and Rosenberg (6) with certain *Hieracia.*

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[1] Somatic segregation is not the only available hypothesis in the cases of the double Matthiola and Petunia. For the double stock *may* have one half or less of its pollen-grains ineffective for fertilization (compare Correns on the pollination of *Mirabilis jalapa,* in *Ber. Deutsch. Bot. Ges.,* Bd. 18, S. 422–435); and in the petunia doubleness *may* be incompletely dominant, as in the greenhouse carnation.

NOTES AND LITERATURE

REPULSION IN WHEAT[1]

THE evidence was furnished by the (F_2) of the cross: Smooth Black × Rough White. "Smooth Black" is a wheat obtained from the (F_2) of the Rivet × Fife cross and it breeds quite true. Its glumes are absolutely glabrous and of a burnished black color. "Rough White" is the well-known Essex Rough Chaff Wheat. The glumes are very hairy and of the ordinary white color. The (F_2) sorted into the following classes:

Rough Black	Rough White	Smooth Black	Smooth White
120	43	47	3

The expectation for the 1:3:3:1 repulsion is:

109.8	49.9	49.3	3.3

"Blackness" is probably not a simple character for in the (F_2) various degrees of it occur—the patches of it on the glumes being of various sizes and intensities of color. There is evidence that it is closely connected with the "gray" color of Rivet glumes.

F. L. ENGLEDOW

THE DETERMINATION OF THE BEST VALUE OF THE COUPLING-RATIO FROM A GIVEN SET OF DATA[1]

MR. G. N. COLLINS has suggested in this journal[2] a general method for determining the value to assign to the coupling-ratio for a given set of data. He has worked out the value of a coefficient of association for the whole series of possible integral ratios 1:1:1:1, 2:1:1:2, etc., and then used the observed value of the same coefficient to decide which ratio gives the best agreement with the facts. The method is very simple, but does not lead to the value which is the most advantageous in a certain sense. If F_1, F_2, F_3, F_4, are the set of theoretical frequencies for a given value of the ratio and if F_1', F_2', F_3', F_4', are the observed fre-

[1] "A Case of Repulsion in Wheat," by F. L. Engledow, St. John's College, Cambridge (Proc. Camb. Phil. Soc., Vol. 18).

[1] F. L. Engledow and G. Udny Yule (Proc. Cambridge Phil. Soc., XVII, 436).

quencies, and if $\chi^2 = \Sigma(F' - F)^2/F$, then the probability p that in random sampling deviations of equal or greater improbability will arise is a function of χ^2 which decreases continually as χ^2 increases. The best value of the ratio will then be that value which makes p a maximum or χ^2 a minimum. The problem taken in the note is to determine this value. Unfortunately the solution is not a simple one, depending on an equation of the fourth degree. A few cases are, however, taken as illustrations and the question of probable error is discussed. The recognized fact that, especially when the coupling-ratio is high, its value may receive considerable alteration without greatly altering the closeness of agreement between theory and fact, receives additional emphasis from some of the results given and makes it clear that considerable caution must be used before attaching importance to the precise values of high ratios.

F. L. E AND G. U. Y.

[2] AM. NAT., XLVI.